L Number	Hits	Search Text	DB	Time stamp
-	94	gaucher with (gene adj therapy)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 07:53
-	43	fabry$ with (gene adj therapy)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 06:44
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-	3	niemann-pick with (gene adj therapy)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 06:45
-	2	gangliosidosis with (gene adj therapy)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 06:45
-	1	sandhoff$ with (gene adj therapy)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 06:45
-	51	(DWEK-RAYMOND-A BUTTERS-TERENCE-D JEYAKUMAR-MYLVAGANAM PLATT-FRANCES-MARY PRIESTMAN-DAVID).in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 07:56
-	1398	gaucher	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 07:56
-	15	((DWEK-RAYMOND-A BUTTERS-TERENCE-D JEYAKUMAR-MYLVAGANAM PLATT-FRANCES-MARY PRIESTMAN-DAVID).in.) and gaucher	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/23 07:56

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NEWS 18 Aug 08 NTIS has been reloaded and enhanced
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NEWS 22 Aug 26 Sequence searching in REGISTRY enhanced
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NEWS 24 Sep 16 Experimental properties added to the REGISTRY file
NEWS 25 Sep 16 Indexing added to some pre-1967 records in CA/CAPLUS
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NEWS 27 Oct 01 CASREACT Enriched with Reactions from 1907 to 1985
NEWS 28 Oct 21 EVENTLINE has been reloaded

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L1 142 GLYCOLIPID STORAGE

=> s gaucher
L2 6343 GAUCHER

=> s l2 and gene therapy
L3 336 L2 AND GENE THERAPY

=> s l3 and review
L4 44 L3 AND REVIEW

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 38 DUP REM L4 (6 DUPLICATES REMOVED)

=> s l5 not py<1999
2 FILES SEARCHED...
L6 17 L5 NOT PY<1999

=> d ti so 1-17

L6 ANSWER 1 OF 17 MEDLINE
TI **Gene therapy** for the lysosomal storage disorders.
SO Curr Opin Mol Ther, (2002 Aug) 4 (4) 349-58.
Journal code: 100891485. ISSN: 1464-8431.

L6 ANSWER 2 OF 17 MEDLINE
TI [Adult **Gaucher** disease].
La maladie de **Gaucher** chez l'adulte.
SO REVUE DE MEDECINE INTERNE, (2001 Dec) 22 Suppl 3 374s-383s. Ref: 20
Journal code: 8101383. ISSN: 0248-8663.

L6 ANSWER 3 OF 17 MEDLINE
TI **Gene therapy** for lysosomal storage disorders.
SO Expert Opin Biol Ther, (2001 Sep) 1 (5) 857-67. Ref: 104
Journal code: 101125414. ISSN: 1471-2598.

L6 ANSWER 4 OF 17 MEDLINE
TI Progress towards hematopoietic stem cell **gene therapy**.
SO Curr Opin Mol Ther, (2000 Aug) 2 (4) 400-11. Ref: 118
Journal code: 100891485. ISSN: 1464-8431.

L6 ANSWER 5 OF 17 MEDLINE
TI **Gaucher**'s disease: a **review** for the internist and hepatologist.
SO HEPATO-GASTROENTEROLOGY, (2000 Jul-Aug) 47 (34) 984-97. Ref: 176
Journal code: 8007849. ISSN: 0172-6390.

L6 ANSWER 6 OF 17 MEDLINE
TI Recombinant proteins for genetic disease.
SO CLINICAL GENETICS, (1999 Jun) 55 (6) 389-94. Ref: 34
Journal code: 0253664. ISSN: 0009-9163.

L6 ANSWER 7 OF 17 MEDLINE
TI Gene transfer approaches to the lysosomal storage disorders.
SO NEUROCHEMICAL RESEARCH, (1999 Apr) 24 (4) 601-15. Ref: 129
Journal code: 7613461. ISSN: 0364-3190.

L6 ANSWER 8 OF 17 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI **Gaucher** disease: Perspectives on a prototype lysosomal disease.
SO CMLS Cellular and Molecular Life Sciences, (April, 2002) Vol. 59, No. 4,
pp. 694-707. http://www.birkhauser.ch/journals/1800/1800_tit.htm. print.
ISSN: 1420-682X.

L6 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI New prospects for the treatment of lysosomal storage diseases
SO Drugs (2002), 62(5), 733-742
CODEN: DRUGAY; ISSN: 0012-6667

L6 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI **Review** in molecular medicine: **gene therapy** of human disease
SO Medicine (Baltimore, MD, United States) (2002), 81(1), 69-86
CODEN: MEDIAV; ISSN: 0025-7974

L6 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI **Gene therapy** by targeting hematopoietic stem cells
SO Saishin Igaku (2001), 56(Sept., Zokango), 2257-2263
CODEN: SAIGAK; ISSN: 0370-8241

L6 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI **Gene therapy** towards **Gaucher** disease
SO Cell (Tokyo, Japan) (2001), 33(10), 388-392
CODEN: SAIBD8; ISSN: 0386-4766

L6 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI Inhibition of substrate synthesis as a strategy for glycolipid lysosomal
storage disease therapy
SO Journal of Inherited Metabolic Disease (2001), 24(2), 275-290
CODEN: JIMDDP; ISSN: 0141-8955

L6 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI Lysosomal storage diseases
SO Carbohydrates in Chemistry and Biology (2000), Volume 4, 945-958.
Editor(s): Ernst, Beat; Hart, Gerald W.; Sinay, Pierre. Publisher: Wiley-VCH Verlag GmbH, Weinheim, Germany.
CODEN: 69AMJE

L6 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI Cerezyme - recombinant protein treatment for **Gaucher**'s disease
SO Journal of Biotechnology (2000), 76(2,3), 259-261
CODEN: JBITD4; ISSN: 0168-1656

L6 ANSWER 16 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI **Gene therapy** for inborn error of metabolism
SO No no Kagaku (1999), 21(11), 1215-1220
CODEN: NNOKFZ; ISSN: 1343-4144

L6 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI Gene and biological therapy for the treatment of cancer
SO Farmacia Hospitalaria (1999), 23(3), 158-169
CODEN: FAHOE2; ISSN: 1130-6343

=> d ti so 15,13,12,10,9,7,3,1

L6 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI Cerezyme - recombinant protein treatment for **Gaucher**'s disease
SO Journal of Biotechnology (2000), 76(2,3), 259-261
CODEN: JBITD4; ISSN: 0168-1656

L6 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI Inhibition of substrate synthesis as a strategy for glycolipid lysosomal
storage disease therapy
SO Journal of Inherited Metabolic Disease (2001), 24(2), 275-290
CODEN: JIMDDP; ISSN: 0141-8955

L6 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI **Gene therapy** towards **Gaucher** disease
SO Cell (Tokyo, Japan) (2001), 33(10), 388-392
CODEN: SAIBD8; ISSN: 0386-4766

L6 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI **Review** in molecular medicine: **gene therapy** of human disease
SO Medicine (Baltimore, MD, United States) (2002), 81(1), 69-86
CODEN: MEDIAV; ISSN: 0025-7974

L6 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2002 ACS
TI New prospects for the treatment of lysosomal storage diseases
SO Drugs (2002), 62(5), 733-742
CODEN: DRUGAY; ISSN: 0012-6667

L6 ANSWER 7 OF 17 MEDLINE
TI Gene transfer approaches to the lysosomal storage disorders.
SO NEUROCHEMICAL RESEARCH, (1999 Apr) 24 (4) 601-15. Ref: 129
Journal code: 7613461. ISSN: 0364-3190.

L6 ANSWER 3 OF 17 MEDLINE
TI **Gene therapy** for lysosomal storage disorders.
SO Expert Opin Biol Ther, (2001 Sep) 1 (5) 857-67. Ref: 104
Journal code: 101125414. ISSN: 1471-2598.

L6 ANSWER 1 OF 17 MEDLINE
TI **Gene therapy** for the lysosomal storage disorders.
SO Curr Opin Mol Ther, (2002 Aug) 4 (4) 349-58.
Journal code: 100891485. ISSN: 1464-8431.

=> d ibib ab 15,13,12,10,9,7,3,1

L6 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:793359 CAPLUS
DOCUMENT NUMBER: 132:330375
TITLE: Cerezyme - recombinant protein treatment for **Gaucher**'s disease
AUTHOR(S): Hoppe, Henry
CORPORATE SOURCE: Genzyme Corporation, Framingham, MA, 01701-9322, USA
SOURCE: Journal of Biotechnology (2000), 76(2,3), 259-261
CODEN: JBITD4; ISSN: 0168-1656
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A **review** with no refs. on the use of recombinant techniques for **gene therapy** of **Gaucher**'s disease.

L6 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:474848 CAPLUS
DOCUMENT NUMBER: 136:209862
TITLE: Inhibition of substrate synthesis as a strategy for

glycolipid lysosomal storage disease therapy
AUTHOR(S): Platt, F. M.; Jeyakumar, M.; Andersson, U.; Priestman,
D. A.; Dwek, R. A.; Butters, T. D.; Cox, T. M.; Lachmann, R. H.; Hollak, C.; Aerts, J. M. F. G.; Van Weely, S.; Hrebicek, M.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A.
CORPORATE SOURCE: Glycobiology Institute, Department of Biochemistry,
University of Oxford, Oxford, UK
SOURCE: Journal of Inherited Metabolic Disease (2001), 24(2),
275-290
CODEN: JIMDDP; ISSN: 0141-8955
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A **review**. The glycosphingolipid (GSL) lysosomal storage diseases are caused by mutations in the genes encoding the glycohydrolases
that catabolize GSLs within lysosomes. In these diseases the substrate
for the defective enzyme accumulates in the lysosome and the stored GSL
leads to cellular dysfunction and disease. The diseases frequently have a
progressive neurodegenerative course. The therapeutic options for treating these diseases are relatively limited, and for the majority there
are no effective therapies. The problem is further compounded by difficulties in delivering therapeutic agents to the brain. Most research
effort to date has focused on strategies for augmenting enzyme levels to
compensate for the underlying defect. These include bone marrow transplantation (BMT), enzyme replacement and **gene therapy**. An alternative strategy that we have been exploring is substrate deprivation. This approach aims to balance the rate of GSL synthesis with the impaired rate of GSL breakdown. The imino sugar N-butyldeoxynojirimycin (NB-DNJ) inhibits the first step in GSL biosynthesis and has been used to evaluate this approach. Studies in an
asymptomatic mouse model of Tay-Sachs disease have shown that substrate
deprivation prevents GSL storage in the CNS. In a severe neurodegenerative mouse model of Sandhoff disease, substrate deprivation
delayed the onset of symptoms and disease progression and significantly
increased life expectancy. Combining NB-DNJ and BMT was found to be
synergistic in the Sandhoff mouse model. A clin. trial in type I **Gaucher** disease has been undertaken and has shown beneficial effects. Efficacy was demonstrated on the basis of significant decreases
in liver and spleen vols., gradual but significant improvement in hematol.
parameters and disease activity markers, together with diminished GSL
biosynthesis and storage as detd. by independent biochem. assays. Further
trials in type I **Gaucher** disease are in progress; studies are planned in patients with GSL storage in the CNS.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:721691 CAPLUS
DOCUMENT NUMBER: 136:379316
TITLE: **Gene therapy** towards **Gaucher** disease
AUTHOR(S): Migita, Makoto
CORPORATE SOURCE: Department of Pediatreics, Department of Biochemistry
and Molecular Biology, Nippon Medical School, Sendagi,
Bunkyo-ku, Tokyo, 113-8602, Japan
SOURCE: Cell (Tokyo, Japan) (2001), 33(10), 388-392
CODEN: SAIBD8; ISSN: 0386-4766
PUBLISHER: Nyu Saiensusha
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A **review**, discussing the problems and potential of **gene therapy** towards **Gaucher** disease.

L6 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:138704 CAPLUS
DOCUMENT NUMBER: 137:179181
TITLE: **Review** in molecular medicine: **gene therapy** of human disease
AUTHOR(S): Balicki, Danuta; Beutler, Ernest
CORPORATE SOURCE: Department of Molecular and Experimental Medicine, The
Scripps Research Institute, La Jolla, CA, 92037, USA
SOURCE: Medicine (Baltimore, MD, United States) (2002), 81(1),
69-86
CODEN: MEDIAV; ISSN: 0025-7974
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A **review** on the development of **gene therapy** which requires the applications of different techniques, specifically designed for the disease being targeted. Both viral and no viral strategies for the delivery of exogenous genetic material to human cells
were developed. The viruses include adenovirus, retrovirus, lentivirus,
adeno-assocd. viruses, Semliki Forest virus, Sindbis virus, vaccinia virus, and SV40. Nonviral strategies used naked DNA, liposomes, proteins,
and peptides, as well as other phys. and chem. means. Targets of **gene therapy** for human disease include **Gaucher** disease, adenosine deaminase deficiency, X-linked severe combined immunodeficiency, cystic fibrosis, cancer, AIDS, cardiovascular disease,
and rheumatoid arthritis. The future of gene transfer depends in the generation of new vectors and the testing of modified vectors in human
trials.
REFERENCE COUNT: 189 THERE ARE 189 CITED REFERENCES AVAILABLE FOR
THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L6 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:419716 CAPLUS
DOCUMENT NUMBER: 137:56839
TITLE: New prospects for the treatment of lysosomal storage diseases
AUTHOR(S): Schiffmann, Raphael; Brady, Roscoe O.
CORPORATE SOURCE: Developmental and Metabolic Neurology Branch, National
Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA
SOURCE: Drugs (2002), 62(5), 733-742
CODEN: DRUGAY; ISSN: 0012-6667
PUBLISHER: Adis International Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A **review**. Although individually rare, lysosomal storage disorders constitute a significant burden on society. To date, enzyme replacement therapy (ERT) has been the most successful therapeutic approach for lysosomal storage disorders. ERT reverses systemic manifestations of **Gaucher** disease but does not effectively treat

the neurol. complications. Recently, ERT produced a redn. of severe neuropathic pain, stabilization of renal disease, and improved vascular function and structure in short-term, placebo-controlled trials in patients with Fabry's disease. Long-term studies are necessary to evaluate the full potential of ERT in this disease. In patients with Pompe disease, a fatal cardiac and skeletal muscle disorder, ERT improved cardiac function and structure, and increased overall muscle strength. It has already increased survival in a small no. of affected infants. ERT also decreased liver and spleen size, joint mobility and quality of life in patients with mucopolysaccharidosis type I, but when the therapeutic protein is administered i.v., it is unlikely to modify the neurol. outcome in this or in other similar disorders. Bone marrow transplantation continues to be effective in **Gaucher** disease, in some forms of mucopolysaccharidosis and in mild forms of Krabbe disease, but it has high morbidity and mortality that limits its use in lysosomal storage disorders. Drugs that slow the rate of formation of accumulating glycolipids are being developed and one of them, OGT-918 (N-butyldeoxynojirimycin), is showing promise in patients with **Gaucher** disease. **Gene therapy** for lysosomal storage disorders holds promise as a replacement for the other therapies described here but requires much more development before clin. efficacy trials.
REFERENCE COUNT: 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 7 OF 17 MEDLINE
ACCESSION NUMBER: 1999242251 MEDLINE
DOCUMENT NUMBER: 99242251 PubMed ID: 10227692
TITLE: Gene transfer approaches to the lysosomal storage disorders.
AUTHOR: Barranger J A; Rice E O; Swaney W P
CORPORATE SOURCE: Human Genetics Department at the University of Pittsburgh, PA 15261, USA.
SOURCE: NEUROCHEMICAL RESEARCH, (1999 Apr) 24 (4) 601-15. Ref: 129
Journal code: 7613461. ISSN: 0364-3190.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990628
Last Updated on STN: 20000303
Entered Medline: 19990617
AB The work summarized in this paper used animal and cell culture models systems to develop **gene therapy** approaches for the lysosomal storage disorders. The results have provided the scientific basis for a clinical trial of gene transfer to hematopoietic stem cells (HSC) in **Gaucher** disease which is now in progress. The clinical experiment is providing evidence of HSC transduction, competitive engraftment of genetically corrected HSC, expression of the GC transgene, and the suggestion of a clinical response. In this paper we will **review** the progress made in **Gaucher** disease and include how gene transfer might be studied in other lysosomal storage disorders.

L6 ANSWER 3 OF 17 MEDLINE
ACCESSION NUMBER: 2001682576 MEDLINE
DOCUMENT NUMBER: 21585480 PubMed ID: 11728220
TITLE: **Gene therapy** for lysosomal storage disorders.
AUTHOR: Barranger J M; Novelli E A
CORPORATE SOURCE: University of Pittsburgh, Department of Human Genetics, PA, USA.. jbarrang@helix.hgen.pitt.edu
SOURCE: Expert Opin Biol Ther, (2001 Sep) 1 (5) 857-67. Ref: 104
Journal code: 101125414. ISSN: 1471-2598.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20011203
Last Updated on STN: 20020823
Entered Medline: 20020822
AB The lysosomal storage disorders (LSD) are monogenic inborn errors of metabolism with heterogeneous pathophysiology and clinical manifestations. In the last decades, these disorders have been models for the development of molecular and cellular therapies for inherited metabolic diseases. Studies in preclinical in vitro systems and animal models have allowed the successful development of bone marrow transplantation (BMT) and enzyme replacement therapy (ERT) as therapeutic options for several LSDs. However, BMT is limited by poor donor availability and high morbidity and mortality, and ERT is not a life-long cure. Moreover, the neuropathology present in many LSDs responded poorly, if at all, to these treatments. Therefore, **gene therapy** is an attractive therapeutic alternative. **Gene therapy** strategies for LSDs have employed ex vivo gene transduction of cellular targets with subsequent transplantation of the enzymatically corrected cells, or direct in vivo delivery of the viral vectors. Oncoretroviral vectors and more recently adeno associated vectors (AAV) and lentiviral vectors have been extensively tested, with some success. This **review** summarises the main **gene therapy** strategies which have been employed or are under development for both non-neurological and neuronopathic LSDs. Some of the in vitro and in vivo preclinical studies presented herein have provided the rationale for a **gene therapy** clinical trial for **Gaucher** disease Type I.

L6 ANSWER 1 OF 17 MEDLINE
ACCESSION NUMBER: 2002463642 IN-PROCESS
DOCUMENT NUMBER: 22210686 PubMed ID: 12222873
TITLE: **Gene therapy** for the lysosomal storage disorders.
AUTHOR: Cabrera-Salazar Mario A; Novelli Enrico; Barranger John A
CORPORATE SOURCE: Department of Human Genetics, University of Pittsburgh, PA 15261, USA.
SOURCE: Curr Opin Mol Ther, (2002 Aug) 4 (4) 349-58.
Journal code: 100891485. ISSN: 1464-8431.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20020912
Last Updated on STN: 20020912
AB The lysosomal storage disorders (LSD) are monogenic inborn errors of metabolism with heterogeneous pathophysiology and clinical manifestations.

In recent decades, these disorders have been models for the development of
molecular and cellular therapies for inherited metabolic diseases. Studies
in preclinical in vitro systems and animal models have established
proof-of-concept for the development of bone marrow transplantation (BMT)
and enzyme-replacement therapy (ERT) as therapeutic options for several
LSDs. BMT is limited by poor donor availability and high morbidity and
mortality, and although ERT is a good treatment, it is not a life-long
cure. Its high cost remains an impediment for developing countries. While
substrate synthesis inhibition therapy is an important idea, its clinical
use is far from certain. The neuropathology present in many LSDs has
responded poorly to BMT or ERT, which makes **gene therapy**
an attractive therapeutic alternative. Oncoretroviral vectors, and more
recently adeno-associated and lentiviral vectors have been tested with
some success. This **review** summarizes the main **gene**
therapy strategies which have been employed or are under
development for both non-neurological and neuronopathic LSDs. Some of the
in vitro and in vivo preclinical studies presented herein have provided
the rationale for **gene therapy** clinical trials for
Gaucher disease Type 1.

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L1 142 S GLYCOLIPID STORAGE
L2 6343 S GAUCHER
L3 336 S L2 AND GENE THERAPY
L4 44 S L3 AND REVIEW
L5 38 DUP REM L4 (6 DUPLICATES REMOVED)
L6 17 S L5 NOT PY<1999

=> s ?butyldeoxygalactonojirimycin
L7 20 ?BUTYLDEOXYGALACTONOJIRIMYCIN

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 15 DUP REM L7 (5 DUPLICATES REMOVED)

=> d ti so 1-15

L8 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS
TI Glucosylceramide synthesis inhibitors for the treatment of brain cancer
SO PCT Int. Appl., 25 pp.
CODEN: PIXXD2

L8 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS
TI N-alkyl deoxygalactonojirimycin derivatives for inhibition of glycolipid
synthesis
SO U.S., 19 pp., Cont.-in-part of U.S. 5,399,567.
CODEN: USXXAM

L8 ANSWER 3 OF 15 MEDLINE
TI Galactonojirimycin derivatives restore mutant human beta-galactosidase
activities expressed in fibroblasts from enzyme-deficient knockout mouse.
SO BRAIN AND DEVELOPMENT, (2001 Aug) 23 (5) 284-7.
Journal code: 7909235. ISSN: 0387-7604.

L8 ANSWER 4 OF 15 MEDLINE
TI Carbohydrate receptor depletion as an antimicrobial strategy for
prevention of urinary tract infection.
SO JOURNAL OF INFECTIOUS DISEASES, (2001 Mar 1) 183 Suppl 1 S70-3.
Journal code: 0413675. ISSN: 0022-1899.

L8 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2002 ACS
TI Use of glucosylceramide synthesis inhibitors in therapy
SO PCT Int. Appl., 31 pp.
CODEN: PIXXD2

L8 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2002 ACS
TI Combination of glucosylceramide synthesis inhibitors and glycolipid
degrading enzyme in therapy
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2

L8 ANSWER 7 OF 15 MEDLINE
TI In vitro inhibition and intracellular enhancement of lysosomal alpha-galactosidase A activity in Fabry lymphoblasts by 1-deoxygalactonojirimycin and its derivatives.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2000 Jul) 267 (13) 4179-86.
Journal code: 0107600. ISSN: 0014-2956.

L8 ANSWER 8 OF 15 MEDLINE DUPLICATE 1
TI N-**butyldeoxygalactonojirimycin**: a more selective inhibitor of glycosphingolipid biosynthesis than N-butyldeoxynojirimycin, in vitro and
in vivo.
SO BIOCHEMICAL PHARMACOLOGY, (2000 Apr 1) 59 (7) 821-9.
Journal code: 0101032. ISSN: 0006-2952.

L8 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI N-butyl-deoxygalactonojirimycin is a more selective compound for the
therapy of glycosphingolipidoses.
SO Journal of Inherited Metabolic Disease, (July, 2000) Vol. 23, No. Supplement 1, pp. 216. print.
Meeting Info.: VIIIth International Conference on Inborn Errors of Metabolism England, Cambridge, UK September 13-17, 2000
ISSN: 0141-8955.

L8 ANSWER 10 OF 15 MEDLINE
TI Accelerated transport and maturation of lysosomal alpha-galactosidase A in
Fabry lymphoblasts by an enzyme inhibitor.
SO NATURE MEDICINE, (1999 Jan) 5 (1) 112-5.
Journal code: 9502015. ISSN: 1078-8956.

L8 ANSWER 11 OF 15 MEDLINE DUPLICATE 2
TI Inhibition of glycosphingolipid biosynthesis does not impair growth or
morphogenesis of the postimplantation mouse embryo.
SO JOURNAL OF NEUROCHEMISTRY, (1998 Feb) 70 (2) 871-82.
Journal code: 2985190R. ISSN: 0022-3042.

L8 ANSWER 12 OF 15 MEDLINE
TI Application of exogenous ceramide to cultured rat spinal motoneurons
promotes survival or death by regulation of apoptosis depending on its
concentrations.
SO JOURNAL OF NEUROSCIENCE RESEARCH, (1998 Nov 15) 54 (4) 475-85.
Journal code: 7600111. ISSN: 0360-4012.

L8 ANSWER 13 OF 15 MEDLINE
TI Evidence that N-linked glycosylation is necessary for hepatitis B virus
secretion.
SO VIROLOGY, (1995 Nov 10) 213 (2) 660-5.
Journal code: 0110674. ISSN: 0042-6822.

L8 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2002 ACS
TI Preparation of N-alkyldeoxygalactonojirimycins as glycolipid biosynthesis
inhibitors.
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2

L8 ANSWER 15 OF 15 MEDLINE DUPLICATE 3
TI N-**butyldeoxygalactonojirimycin** inhibits glycolipid biosynthesis but does not affect N-linked oligosaccharide processing.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Oct 28) 269 (43) 27108-14.
Journal code: 2985121R. ISSN: 0021-9258.

=> d his

(FILE 'HOME' ENTERED AT 06:47:12 ON 23 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 06:47:36 ON 23 OCT 2002
L1 142 S GLYCOLIPID STORAGE
L2 6343 S GAUCHER
L3 336 S L2 AND GENE THERAPY
L4 44 S L3 AND REVIEW
L5 38 DUP REM L4 (6 DUPLICATES REMOVED)
L6 17 S L5 NOT PY<1999
L7 20 S ?BUTYLDEOXYGALACTONOJIRIMYCIN
L8 15 DUP REM L7 (5 DUPLICATES REMOVED)

=> d his

(FILE 'HOME' ENTERED AT 06:47:12 ON 23 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 06:47:36 ON 23 OCT 2002
L1 142 S GLYCOLIPID STORAGE
L2 6343 S GAUCHER
L3 336 S L2 AND GENE THERAPY
L4 44 S L3 AND REVIEW
L5 38 DUP REM L4 (6 DUPLICATES REMOVED)
L6 17 S L5 NOT PY<1999
L7 20 S ?BUTYLDEOXYGALACTONOJIRIMYCIN
L8 15 DUP REM L7 (5 DUPLICATES REMOVED)

=> log hold
COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 66.74 66.95

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)
SINCE FILE TOTAL
ENTRY SESSION
CA SUBSCRIBER PRICE -3.10 -3.10

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 07:08:12 ON 23 OCT 2002

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1636DXS

PASSWORD:
* * * * * * RECONNECTED TO STN INTERNATIONAL * * * * * *
SESSION RESUMED IN FILE 'MEDLINE, BIOSIS, CAPLUS' AT 07:12:45 ON 23 OCT 2002
FILE 'MEDLINE' ENTERED AT 07:12:45 ON 23 OCT 2002
FILE 'BIOSIS' ENTERED AT 07:12:45 ON 23 OCT 2002
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FILE 'CAPLUS' ENTERED AT 07:12:45 ON 23 OCT 2002
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COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 66.74 66.95

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)
SINCE FILE TOTAL
ENTRY SESSION
CA SUBSCRIBER PRICE -3.10 -3.10

=> sandhoff mouse or sandhoff mice
SANDHOFF IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system. For a list of commands available to you in the current file, enter "HELP COMMANDS" at an arrow prompt (=>).

=> s sandhoff mouse or sandhoff mice
L9 15 SANDHOFF MOUSE OR SANDHOFF MICE

=> dup rem l15
L15 IS NOT VALID HERE
The L-number entered has not been defined in this session, or it has been deleted. To see the L-numbers currently defined in this session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10 9 DUP REM L9 (6 DUPLICATES REMOVED)

=> d ti so 1-9

L10 ANSWER 1 OF 9 MEDLINE DUPLICATE 1
TI Lysosomal storage results in impaired survival but normal neurite outgrowth in dorsal root ganglion neurones from a mouse model of Sandhoff
disease.
SO NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY, (2002 Feb) 28 (1) 23-34.
Journal code: 7609829. ISSN: 0305-1846.

L10 ANSWER 2 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Lysosomal storage results in impaired survival but normal neurite outgrowth in dorsal root ganglion neurons from a mouse model of Sandhoff
disease.
SO Society for Neuroscience Abstracts, (2001) Vol. 27, No. 2, pp. 2624.
print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San
Diego, California, USA November 10-15, 2001
ISSN: 0190-5295.

L10 ANSWER 3 OF 9 MEDLINE DUPLICATE 2
TI Substrate reduction therapy for glycosphingolipid storage disorders.
SO EXPERT OPINION ON INVESTIGATIONAL DRUGS, (2001 Mar) 10 (3) 455-66. Ref:
59
Journal code: 9434197. ISSN: 1354-3784.

L10 ANSWER 4 OF 9 MEDLINE DUPLICATE 3
TI Inhibition of substrate synthesis as a strategy for glycolipid lysosomal
storage disease therapy.
SO JOURNAL OF INHERITED METABOLIC DISEASE, (2001 Apr) 24 (2) 275-90. Ref: 45
Journal code: 7910918. ISSN: 0141-8955.

L10 ANSWER 5 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

TI Electroneuropathology of Sandhoff's disease in mice.
SO Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 263. print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San
Diego, California, USA November 10-15, 2001
ISSN: 0190-5295.

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2002 ACS
TI Targeting the hexosaminidase genes: mouse models of the GM2 gangliosidoses
SO Advances in Genetics (2001), 44(Tay-Sachs Disease), 225-231
CODEN: ADGEAV; ISSN: 0065-2660

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2002 ACS
TI Combination of glucosylceramide synthesis inhibitors and glycolipid
degrading enzyme in therapy
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2

L10 ANSWER 8 OF 9 MEDLINE DUPLICATE 4
TI Apoptotic cell death in mouse models of GM2 gangliosidosis and observations on human Tay-Sachs and Sandhoff diseases.
SO HUMAN MOLECULAR GENETICS, (1997 Oct) 6 (11) 1879-85.
Journal code: 9208958. ISSN: 0964-6906.

L10 ANSWER 9 OF 9 MEDLINE
TI [Genetic study of GM2 gangliosidosis (Tay-Sachs and Sandhoff) by the study
of the hexosaminidases of the Sandhoff-rodents hybrids (mouse and hamster)].
Contribution a l'etude genetique des gangliosidoses GM2 (Tay-Sachs et
Sandhoff) par l'etude des hexosaminidases des hybrides Sandhoff-rongeurs
(souris et hamster).
SO ANNALES DE GENETIQUE, (1975 Sep) 18 (3) 163-8.
Journal code: 0370562. ISSN: 0003-3995.

=> d ibib ab 1

L10 ANSWER 1 OF 9 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002117701 MEDLINE
DOCUMENT NUMBER: 21839719 PubMed ID: 11849560
TITLE: Lysosomal storage results in impaired survival but normal
neurite outgrowth in dorsal root ganglion neurones from a mouse model of Sandhoff disease.
AUTHOR: Sango K; Yamanaka S; Ajiki K; Tokashiki A; Watabe K
CORPORATE SOURCE: Department of Developmental Morphology, Tokyo Metropolitan
Institute for Neuroscience, Fuchu-shi, Tokyo, Japan..
kazsango@tmin.ac.jp
SOURCE: NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY, (2002 Feb) 28 (1)
23-34.
Journal code: 7609829. ISSN: 0305-1846.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020220
Last Updated on STN: 20020401
Entered Medline: 20020326
AB Sandhoff disease is a heritable lysosomal storage disease resulting from
impaired degradation of GM2 ganglioside and related substrates. A mouse
model of Sandhoff disease created by gene targeting displays progressive
neurological manifestations, similar to patients with the disease. In the
present in vivo and in vitro studies, we examined morphological and functional abnormalities of dorsal root ganglion (DRG) neurones in Sandhoff disease mice at an asymptomatic stage (approximately 1 month of
age). Light microscopic studies with Nissl staining and immunocytochemistry suggested extensive intracytoplasmic storage of GM2
ganglioside in the **Sandhoff mouse** DRG neurones. These findings were consistent with the results of electron microscopy, in which
a huge number of pleomorphic inclusion bodies immunoreactive for GM2
ganglioside were present in the cytoplasm of the neurones. The inclusion
bodies were also identified in satellite cells and Schwann cells in the **Sandhoff mouse** DRG. The survival ratios of DRG neurones after 1, 2, 4 and 6 days in culture were significantly lower in the **Sandhoff mice** than in the age-matched heterozygous mice. The ratio of neurite-bearing cells on poly-l-lysine-coated dishes after 2
days in culture was also lower by approximately 10% in the **Sandhoff mice** compared to the heterozygotes, but additional coating of laminin onto poly-l-lysine dramatically enhanced the
neurite extension from the neurones in both groups of mice. These results
indicate that accumulation of GM2 ganglioside in DRG neurones impairs the
capability of the neurones to survive in vitro, although viable neurones
from the **Sandhoff mice** in culture can regenerate neurites nearly as well as unaffected neurones.

=> d his

(FILE 'HOME' ENTERED AT 06:47:12 ON 23 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 06:47:36 ON 23 OCT 2002
L1 142 S GLYCOLIPID STORAGE
L2 6343 S GAUCHER
L3 336 S L2 AND GENE THERAPY
L4 44 S L3 AND REVIEW
L5 38 DUP REM L4 (6 DUPLICATES REMOVED)
L6 17 S L5 NOT PY<1999
L7 20 S ?BUTYLDEOXYGALACTONOJIRIMYCIN
L8 15 DUP REM L7 (5 DUPLICATES REMOVED)
L9 15 S SANDHOFF MOUSE OR SANDHOFF MICE
L10 9 DUP REM L9 (6 DUPLICATES REMOVED)

=> log hold
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 81.64 81.85

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)
SINCE FILE ENTRY TOTAL SESSION
CA SUBSCRIBER PRICE -3.10 -3.10

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 07:15:44 ON 23 OCT 2002

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

STNLOGON timed out

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:
SSSPTA1636DXS
LOGINID:
PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

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NEWS 3 Apr 09 BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4 Apr 09 ZDB will be removed from STN
NEWS 5 Apr 19 US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6 Apr 22 Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7 Apr 22 BIOSIS Gene Names now available in TOXCENTER
NEWS 8 Apr 22 Federal Research in Progress (FEDRIP) now available
NEWS 9 Jun 03 New e-mail delivery for search results now available
NEWS 10 Jun 10 MEDLINE Reload
NEWS 11 Jun 10 PCTFULL has been reloaded
NEWS 12 Jul 02 FOREGE no longer contains STANDARDS file segment
NEWS 13 Jul 22 USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14 Jul 29 Enhanced polymer searching in REGISTRY
NEWS 15 Jul 30 NETFIRST to be removed from STN
NEWS 16 Aug 08 CANCERLIT reload
NEWS 17 Aug 08 PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18 Aug 08 NTIS has been reloaded and enhanced
NEWS 19 Aug 19 Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20 Aug 19 IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21 Aug 19 The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22 Aug 26 Sequence searching in REGISTRY enhanced
NEWS 23 Sep 03 JAPIO has been reloaded and enhanced
NEWS 24 Sep 16 Experimental properties added to the REGISTRY file
NEWS 25 Sep 16 Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 26 Sep 16 CA Section Thesaurus available in CAPLUS and CA
NEWS 27 Oct 01 CASREACT Enriched with Reactions from 1907 to 1985
NEWS 28 Oct 21 EVENTLINE has been reloaded

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CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP),
AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
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FILE 'HOME' ENTERED AT 08:43:32 ON 23 OCT 2002

=> file medline biosis caplus
COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 08:43:43 ON 23 OCT 2002

FILE 'BIOSIS' ENTERED AT 08:43:43 ON 23 OCT 2002
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=> s metabol(s)disease
L1 2 METABOL(S) DISEASE

=> s metabol?(s)disease
L2 225502 METABOL?(S) DISEASE

=> s combin?(2a)therap?
L3 203924 COMBIN?(2A) THERAP?

=> s l2 and l3
L4 1930 L2 AND L3

=> s compilcat?
L5 2 COMPILCAT?

=> s complicat?
L6 1676579 COMPLICAT?

=> s l4 and l6
L7 384 L4 AND L6

=> s l7 and review
L8 47 L7 AND REVIEW

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9 44 DUP REM L8 (3 DUPLICATES REMOVED)

=> d ti so 1-44

L9 ANSWER 1 OF 44 MEDLINE
TI Drug treatment of non-motor symptoms in Parkinson's disease.
SO Expert Opin Pharmacother, (2002 Apr) 3 (4) 381-8.
Journal code: 100897346. ISSN: 1465-6566.

L9 ANSWER 2 OF 44 CAPLUS COPYRIGHT 2002 ACS
TI Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency
SO Annals of Internal Medicine (2002), 136(4), 320-334
CODEN: AIMEAS; ISSN: 0003-4819

L9 ANSWER 3 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Heterogeneity affecting outcome from acute stroke therapy: Making reperfusion worse.
SO Stroke, (October, 2001) Vol. 32, No. 10, pp. 2318-2327. print.
ISSN: 0039-2499.

L9 ANSWER 4 OF 44 MEDLINE
TI Step care therapy for hypertension in diabetic patients.
SO MAYO CLINIC PROCEEDINGS, (2001 Dec) 76 (12) 1266-74. Ref: 48
Journal code: 0405543. ISSN: 0025-6196.

L9 ANSWER 5 OF 44 MEDLINE DUPLICATE 1
TI Matching treatment to pathophysiology in type 2 diabetes.
SO CLINICAL THERAPEUTICS, (2001 May) 23 (5) 646-59; discussion 645. Ref: 65
Journal code: 7706726. ISSN: 0149-2918.

L9 ANSWER 6 OF 44 MEDLINE
TI Gycemic control, mealtime glucose excursions, and diabetic **complications** in type 2 diabetes mellitus.
SO MAYO CLINIC PROCEEDINGS, (2001 Jun) 76 (6) 609-18. Ref: 123
Journal code: 0405543. ISSN: 0025-6196.

L9 ANSWER 7 OF 44 MEDLINE
TI The metabolic toxicities of antiretroviral therapy.
SO INTERNATIONAL JOURNAL OF STD AND AIDS, (2001 Sep) 12 (9) 555-62; quiz
563-4. Ref: 71
Journal code: 9007917. ISSN: 0956-4624.

L9 ANSWER 8 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Current therapies and emerging targets for the treatment of diabetes.
SO Current Pharmaceutical Design, (April, 2001) Vol. 7, No. 6, pp. 417-450.
print.
ISSN: 1381-6128.

L9 ANSWER 9 OF 44 CAPLUS COPYRIGHT 2002 ACS
TI Disturbances of essential fatty acid metabolism in neural **complications** of diabetes
SO Fatty Acids (2001), 239-256. Editor(s): Mostofsky, David I.; Yehuda,
Shlomo; Salem, Norman, Jr. Publisher: Humana Press Inc., Totowa, N. J.
CODEN: 69CATW

L9 ANSWER 10 OF 44 MEDLINE
TI [Treatment of hypertension in obesity].
Behandlung der Hypertonie bei Adipositas.
SO HERZ, (2001 May) 26 (3) 209-21. Ref: 109
Journal code: 7801231. ISSN: 0340-9937.

L9 ANSWER 11 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
TI Metabolic **complications** associated with antiretroviral therapy.
SO Antiviral Research, (September, 2001) Vol. 51, No. 3, pp. 151-177. print.
ISSN: 0166-3542.

L9 ANSWER 12 OF 44 MEDLINE
TI Hypertriglyceridemia: a **review** of clinical relevance and treatment options: focus on cerivastatin.
SO CURRENT MEDICAL RESEARCH AND OPINION, (2001) 17 (1) 60-73. Ref: 88
Journal code: 0351014. ISSN: 0300-7995.

L9 ANSWER 13 OF 44 MEDLINE
TI Delavirdine: a **review** of its use in HIV infection.
SO DRUGS, (2000 Dec) 60 (6) 1411-44. Ref: 117
Journal code: 7600076. ISSN: 0012-6667.

L9 ANSWER 14 OF 44 MEDLINE
TI Nevirapine: a **review** of its use in the prevention and treatment of paediatric HIV infection.
SO PAEDIATRIC DRUGS, (2000 Sep-Oct) 2 (5) 373-407. Ref: 130
Journal code: 100883685. ISSN: 1174-5878.

L9 ANSWER 15 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Unrelated placental/umbilical cord blood cell (UCBC) transplantation in
children with high-risk sickle cell disease (SCD.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 366b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology
San Francisco, California, USA December 01-05, 2000 American Society of
Hematology
. ISSN: 0006-4971.

L9 ANSWER 16 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Hemorrhagic pyelitis, ureteritis, and cystitis secondary to cyclophosphamide: Case report and **review** of the literature.
SO Gynecologic Oncology, (Feb., 2000) Vol. 76, No. 2, pp. 223-225.
ISSN: 0090-8258.

L9 ANSWER 17 OF 44 MEDLINE
TI Recognition and management of depression in primary care: a focus on the
elderly. A pharmacotherapeutic overview of the selection process among the
traditional and new antidepressants.
SO AMERICAN JOURNAL OF THERAPEUTICS, (2000 May) 7 (3) 205-26. Ref: 143
Journal code: 9441347. ISSN: 1075-2765.

L9 ANSWER 18 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Challenges in the dietary treatment of cystic fibrosis related diabetes mellitus.
SO Clinical Nutrition (Edinburgh), (April, 2000) Vol. 19, No. 2, pp. 87-93.
print.
ISSN: 0261-5614.

L9 ANSWER 19 OF 44 MEDLINE
TI **Review** of prandial glucose regulation with repaglinide: a solution to the problem of hypoglycaemia in the treatment of Type 2 diabetes?.
SO INTERNATIONAL JOURNAL OF OBESITY AND RELATED METABOLIC DISORDERS, (2000
Sep) 24 Suppl 3 S21-31. Ref: 81
Journal code: 9313169. ISSN: 0307-0565.

L9 ANSWER 20 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI New directions in type 2 diabetes mellitus: An update of current oral antidiabetic therapy.
SO Journal of the National Medical Association, (July, 1999) Vol. 91, No. 7,
pp. 389-395.
ISSN: 0027-9684.

L9 ANSWER 21 OF 44 MEDLINE
TI Bartter's syndrome in Arabic children: **review** of 13 cases.
SO PEDIATRICS INTERNATIONAL, (1999 Jun) 41 (3) 299-303.
Journal code: 100886002. ISSN: 1328-8067.

L9 ANSWER 22 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

TI A **review** of the results from the UKPDS: Answers or questions.
SO Acta Diabetologica., (Sept., 1999) Vol. 36, No. Suppl. 1-2, pp. S18-S24.
ISSN: 0940-5429.

L9 ANSWER 23 OF 44 MEDLINE
TI Long-term survival in rhinocerebral mucormycosis. Case report.
SO JOURNAL OF NEUROSURGERY, (1998 Mar) 88 (3) 570-5. Ref: 43
Journal code: 0253357. ISSN: 0022-3085.

L9 ANSWER 24 OF 44 MEDLINE
TI Pharmacological treatment of wounds.
SO SEMINARS IN CUTANEOUS MEDICINE AND SURGERY, (1998 Dec) 17 (4) 260-5. Ref: 47
Journal code: 9617260. ISSN: 1085-5629.

L9 ANSWER 25 OF 44 MEDLINE
TI New antiretrovirals and new combinations.
SO AIDS, (1998) 12 Suppl A S165-74. Ref: 103
Journal code: 8710219. ISSN: 0269-9370.

L9 ANSWER 26 OF 44 MEDLINE
TI Pathophysiology and treatment of lipid perturbation after cardiac transplantation.
SO CURRENT OPINION IN CARDIOLOGY, (1997 Mar) 12 (2) 153-60. Ref: 79
Journal code: 8608087. ISSN: 0268-4705.

L9 ANSWER 27 OF 44 MEDLINE
TI Hereditary hemochromatosis: recent advances in molecular genetics and clinical management.
SO HAEMATOLOGICA, (1997 Jan-Feb) 82 (1) 77-84. Ref: 94
Journal code: 0417435. ISSN: 0390-6078.

L9 ANSWER 28 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Acarbose: A **review** of US clinical experience.
SO Clinical Therapeutics, (1997) Vol. 19, No. 1, pp. 16-26.
ISSN: 0149-2918.

L9 ANSWER 29 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Diuretics in liver cirrhosis with ascites.
SO Verdauungskrankheiten, (1996) Vol. 14, No. 4, pp. 136-145.
ISSN: 0174-738X.

L9 ANSWER 30 OF 44 MEDLINE
TI [Use of parenteral feeding in the multimodality treatment of Crohn's disease and nonspecific ulcerative colitis].
Primenenie parenteral'nogo pitaniia v kompleksnom lechenii bolezni Krona i nespetsificheskogo iazvennogo kolita.
SO VESTNIK ROSSIISKOI AKADEMII MEDITSINSKIKH NAUK, (1996) (9) 40-4. Ref: 58
Journal code: 9215641. ISSN: 0869-6047.

L9 ANSWER 31 OF 44 MEDLINE
TI [Nontraumatic coma in extramural practice].
Le coma non traumatique en pratique extrahospitaliere.
SO SCHWEIZERISCHE RUNDSCHAU FUR MEDIZIN PRAXIS, (1995 Nov 7) 84 (45) 1321-30.
Ref: 16
Journal code: 8403202. ISSN: 1013-2058.

L9 ANSWER 32 OF 44 MEDLINE DUPLICATE 3
TI Rifabutin-associated uveitis.
SO ANNALS OF PHARMACOTHERAPY, (1995 Nov) 29 (11) 1149-55. Ref: 45
Journal code: 9203131. ISSN: 1060-0280.

L9 ANSWER 33 OF 44 MEDLINE
TI Rimantadine: a clinical perspective.
SO ANNALS OF PHARMACOTHERAPY, (1995 Mar) 29 (3) 299-310. Ref: 87
Journal code: 9203131. ISSN: 1060-0280.

L9 ANSWER 34 OF 44 MEDLINE
TI Antihyperglycaemic agents. Drug interactions of clinical importance.
SO DRUG SAFETY, (1995 Jan) 12 (1) 32-45. Ref: 124
Journal code: 9002928. ISSN: 0114-5916.

L9 ANSWER 35 OF 44 CAPLUS COPYRIGHT 2002 ACS
TI Interaction studies in **metabolic diseases**
SO Klinische Pharmakologie (1994), 11(Drug Interaction Studies during Drug Development), 38-42
CODEN: KLPHEH; ISSN: 0937-0978

L9 ANSWER 36 OF 44 MEDLINE
TI Antiepileptic drugs. A **review** of clinically significant drug interactions.
SO DRUG SAFETY, (1993 Sep) 9 (3) 156-84. Ref: 303
Journal code: 9002928. ISSN: 0114-5916.

L9 ANSWER 37 OF 44 MEDLINE
TI [The combined use of alpha-adrenoblockaders and calcium antagonists in the etiopathogenetic therapy of cardiovascular diseases].
Kompleksnoe ispol'zovanie al'fa-adrenoblokatorov i antagonistov kal'tsiia v etiopatogeneticheskoi terapii serdechno-sosudistykh zabolevanii.
SO EKSPERIMENTALNAIA I KLINICHESKAIA FARMAKOLOGIIA, (1993 Jul-Aug) 56 (4) 26-30. Ref: 116
Journal code: 9215981. ISSN: 0869-2092.

L9 ANSWER 38 OF 44 MEDLINE
TI Protein calorie malnutrition and cancer therapy.
SO DRUG SAFETY, (1992 Nov-Dec) 7 (6) 404-16. Ref: 91
Journal code: 9002928. ISSN: 0114-5916.

L9 ANSWER 39 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI EXERCISE TRAINING IN OBESE DIABETIC PATIENTS SPECIAL CONSIDERATIONS.
SO SPORTS MED, (1992) 14 (3), 171-189.
CODEN: SPMEE7. ISSN: 0112-1642.

L9 ANSWER 40 OF 44 MEDLINE
TI Insulin resistance and essential hypertension: pathophysiologic and therapeutic implications.
SO JOURNAL OF HYPERTENSION. SUPPLEMENT, (1992 Apr) 10 (2) S9-15. Ref: 85
Journal code: 8501422. ISSN: 0952-1178.

L9 ANSWER 41 OF 44 MEDLINE
TI Skeletal involvement in children who have chronic granulomatous disease.
SO JOURNAL OF BONE AND JOINT SURGERY. AMERICAN VOLUME, (1991 Jan) 73 (1) 37-51.
Journal code: 0014030. ISSN: 0021-9355.

L9 ANSWER 42 OF 44 MEDLINE
TI The effects of antihypertensive agents on serum lipids and lipoproteins.
SO ARCHIVES OF INTERNAL MEDICINE, (1988 Jun) 148 (6) 1280-8. Ref: 202
Journal code: 0372440. ISSN: 0003-9926.

L9 ANSWER 43 OF 44 MEDLINE
TI The diuretic dilemma and the management of mild hypertension.
SO JOURNAL OF CLINICAL HYPERTENSION, (1986 Jun) 2 (2) 195-202. Ref: 28

Journal code: 8510461. ISSN: 0748-450X.

L9 ANSWER 44 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI SEX HORMONES AND RELATED COMPOUNDS INCLUDING ORAL CONTRACEPTIVES.
SO DUKES, M. N. G. (ED.). SIDE EFFECTS OF DRUGS ANNUAL, VOL. 4. A WORLDWIDE
YEARLY SURVEY OF NEW DATA AND TRENDS. XVIII+376P. EXCERPTA MEDICA:
AMSTERDAM, NETHERLANDS (DIST. IN USA BY ELSEVIER/NORTH HOLLAND, INC.: NEW
YORK, N.Y.). (1980) 0 (0), P275-293.
CODEN: SEDAD8. ISSN: 0378-6080. ISBN: 90-219-3053-6, 0-444-90130-2.

=> d ibib ab 36,35,34,12,7,2

L9 ANSWER 36 OF 44 MEDLINE
ACCESSION NUMBER: 94059420 MEDLINE
DOCUMENT NUMBER: 94059420 PubMed ID: 8240723
TITLE: Antiepileptic drugs. A **review** of clinically significant drug interactions.
AUTHOR: Patsalos P N; Duncan J S
CORPORATE SOURCE: University Department of Clinical Neurology, Institute of Neurology, London, England.
SOURCE: DRUG SAFETY, (1993 Sep) 9 (3) 156-84. Ref: 303
Journal code: 9002928. ISSN: 0114-5916.
PUB. COUNTRY: New Zealand
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 19940201
Entered Medline: 19931223
AB Approximately 20 to 30% of patients with active intractable epilepsy are commonly treated with polytherapy antiepileptic drug regimens, and these patients may experience **complicated** drug interactions. Furthermore, because of the long term nature of treatment, the possibility of drug interactions with drugs used for the treatment of concomitant **disease** is high. Classically, clinically significant drug interactions, both pharmacokinetic and pharmacodynamic, have been considered to be detrimental to the patient, necessitating dosage adjustment. However, this need not always be the case. With the introduction of new drugs (e.g. vigabatrin and lamotrigine) with known mechanisms of action, the possibility exists that these can be used synergistically. The most commonly observed clinically significant pharmacokinetic interactions can be attributed to interactions at the **metabolic** and serum protein binding levels. The best known examples relate to induction (e.g. phenobarbital, phenytoin, carbamazepine and primidone) or inhibition [e.g. valproic acid (sodium valproate)] of hepatic monoxygenase enzymes. The extent and direction of interactions between the different antiepileptic drugs are varied and unpredictable. Interactions in which the **metabolism** of phenobarbital, phenytoin or carbamazepine is inhibited are particularly important since these are commonly associated with toxicity. Some inhibitory drugs include macrolide antibiotics, chloramphenicol, cimetidine, isoniazid and numerous sulphonamides. A reduction in efficacy of antibiotic, cardiovascular, corticosteroid, oral anticoagulant and oral contraceptive drugs occurs during **combination therapy** with enzyme-inducing antiepileptic drugs. Discontinuation of the enzyme inducer or inhibitor will influence the concentrations of the remaining drug(s) and may necessitate dosage readjustment.

L9 ANSWER 35 OF 44 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:877491 CAPLUS
DOCUMENT NUMBER: 124:29
TITLE: Interaction studies in **metabolic diseases**
AUTHOR(S): Latocha, G.
CORPORATE SOURCE: Institute Clinical Pharmacology International, Bayer AG, Wuppertal, Germany
SOURCE: Klinische Pharmakologie (1994), 11(Drug Interaction Studies during Drug Development), 38-42
CODEN: KLPHEH; ISSN: 0937-0978
PUBLISHER: W. Zuckschwerdt Verlag
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A **review** with 12 refs. Diabetes mellitus and hyperlipoproteinemia are two clin. entities with profound metabolic abnormalities. Patients afflicted with a **metabolic disease** usually require life-long treatment. They are often treated with a **combination therapy**. Moreover, **complications** of the primary disease frequently require addnl. pharmacol. agents. For these reasons both groups of drugs, i.e. for the treatment of primary and secondary disease, have to be considered as potential interaction partners during the development of a new pharmacol. agent for the indications discussed.

L9 ANSWER 34 OF 44 MEDLINE
ACCESSION NUMBER: 95260440 MEDLINE
DOCUMENT NUMBER: 95260440 PubMed ID: 7741982
TITLE: Antihyperglycaemic agents. Drug interactions of clinical importance.
AUTHOR: Scheen A J; Lefebvre P J
CORPORATE SOURCE: Department of Medicine, CHU Sart Tilman, Liege, Belgium.
SOURCE: DRUG SAFETY, (1995 Jan) 12 (1) 32-45. Ref: 124
Journal code: 9002928. ISSN: 0114-5916.
PUB. COUNTRY: New Zealand
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950621
Last Updated on STN: 19950621
Entered Medline: 19950615
AB Non-insulin-dependent (type 2) diabetes mellitus (NIDDM) affects middle-aged or elderly people who frequently have several other concomitant **diseases**, especially obesity, hypertension, dyslipidaemias, coronary insufficiency, heart failure and arthropathies. Thus, polymedication is the rule in this population, and the risk of drug interactions is important, particularly in elderly patients. The present **review** is restricted to the interactions of other drugs with antihyperglycaemic compounds, and will not consider the mirror image, i.e. the interactions of antihyperglycaemic agents with other drugs. Oral antihyperglycaemic agents include sulphonylureas, biguanides--essentially metformin since the withdrawn of phenformin and buformin--and alpha-glucosidase inhibitors, acarbose being the only representative on the market. These drugs can be used alone or in combination to

obtain
better **metabolic** control, sometimes with insulin. Drug interactions with antihyperglycaemic agents can be divided into pharmacokinetic and pharmacodynamic interactions. Most pharmacokinetic
studies concern sulphonylureas, whose action may be enhanced by numerous
other drugs, thus increasing the risk of hypoglycaemia. Such an effect may
result essentially from protein binding displacement, inhibition of hepatic **metabolism** and reduction of renal clearance. Reduction of the hypoglycaemic activity of sulphonylureas due to pharmacokinetic
interactions with other drugs appears to be much less frequent. Drug interactions leading to an increase in plasma metformin concentrations,
mainly by reducing the renal excretion or the hepatic **metabolism** of the biguanide, should be avoided to limit the risk of hyperlactaemia.
Owing to its mode of action, pharmacokinetic interferences with acarbose
are limited to the gastrointestinal tract, but have not been extensively studied yet. Pharmacodynamic interactions are quite numerous and may
result in a potentiation of the hypoglycaemic action or, conversely, in a
deterioration of blood glucose control. Such interactions may be observed
whatever the type of antidiabetic treatment. They result from the intrinsic properties of the coprescribed drug on insulin secretion and action, or on a key step of carbohydrate **metabolism**. Finally, a combination of 2 to 3 antihyperglycaemic agents is common for treating
patients with NIDDM to benefit from the synergistic effect of compounds
acting on different sites of carbohydrate **metabolism**. Possible pharmacokinetic interactions between alpha-glucosidase inhibitors and
classical antidiabetic oral agents should be better studied in the diabetic population.

L9 ANSWER 12 OF 44 MEDLINE
ACCESSION NUMBER: 2002022992 MEDLINE
DOCUMENT NUMBER: 21358569 PubMed ID: 11464448
TITLE: Hypertriglyceridemia: a **review** of clinical relevance and treatment options: focus on cerivastatin.
AUTHOR: Breuer H W
CORPORATE SOURCE: Abteilung fur Innere Medizin, St. Carolus-Krankenhaus
Gorlitz, Carolusstr. 212, 02827 Gorlitz, Germany..
breuer.carolus@gmx.de
SOURCE: CURRENT MEDICAL RESEARCH AND OPINION, (2001) 17 (1) 60-73.
Ref: 88
Journal code: 0351014. ISSN: 0300-7995.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20020121
Last Updated on STN: 20020121
Entered Medline: 20011204
AB The triglyceride (TG) level is one of several lipid parameters that can
aid prediction of coronary heart **disease** (CHD) risk. An elevated plasma TG level is strongly associated with an increased risk of CHD.
Hypertriglyceridemia, the second most common dyslipidemic abnormality in
hypertensive subjects after increased low-density lipoprotein cholesterol
(LDL-C), is defined by the National Cholesterol Education Programme (NCEP)
as a fasting TG level of > 2.26 mmol/l (> 200 mg/dl) and is recognised as
a primary indicator for treatment in type IIb dyslipidemia. Raised TG levels can be present in individuals at risk for CHD when the total cholesterol is normal. However, not all individuals with raised TG levels
have increased risk of CHD. Factors such as: diet, age, lifestyle, and a range of medical conditions, drug therapy and **metabolic** disorders, can all affect the TG level. In some of these circumstances, other factors protect against the risk of CHD, and can minimise or negate
the effect of the risk factors present. Although TG reducing therapy has
been shown to be associated with an improved clinical outcome, more
research is needed to determine whether this is an independent effect of
TG reduction or an effect of normalising the overall lipid profile in hypertriglyceridemic patients. Further trials are required to quantify the
clinical benefits of lowering TG to 'target' levels and to confirm targets
defined by NCEP-II (shown in Table 1). The role of TG in CHD pathogenesis
is thought to involve several direct and indirect mechanisms, such as effects on the **metabolism** of other lipoproteins, transport proteins, enzymes, and on coagulation and endothelial dysfunction. More
research is required to fully elucidate the role of TG, the ways in which
it can influence other risk factors and the mechanism of its own more direct role in the atherogenic process. Patients with hypertriglyceridemia
have been shown to respond well to dietary control and to the use of lipid
lowering drugs such as 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG CoA)
reductase inhibitors (known as statins), fibrates and nicotinic acids. However, recent retrospective real-life clinical studies show that only 38% of patients receiving some form of lipid-lowering therapy achieved
NCEP-defined LDL-C target levels, demonstrating the need for the use of
more aggressive treatment. In hypertriglyceridemic patients, the newer
statins, cerivastatin and atorvastatin, have shown comparable efficacy in
reducing TG compared with the older statins. Achieving NCEP target lipid
levels has been shown to reduce the risk of cardiovascular **disease** in dyslipidemic individuals, including high-risk patient groups such as
those with additional risk factors, existing heart **disease**, diabetes mellitus and **metabolic** syndrome. Although the latest clinical studies investigating **combination therapies**, i.e. dual therapy with both a statin and a fibrate, have demonstrated them
to be effective for overall control of lipid parameters and reducing coronary events, it is not yet clear whether this offers any significant advantage over monotherapy. Results from ongoing longer-term end-point
clinical studies may provide further information in this area and consequent **reviews** of primary care management policies for dyslipidemia. Statin monotherapy may be a reliable option for primary care
treatment of dyslipidemia (including hypertriglyceridemia).

L9 ANSWER 7 OF 44 MEDLINE
ACCESSION NUMBER: 2001471993 MEDLINE
DOCUMENT NUMBER: 21407977 PubMed ID: 11516363
TITLE: The metabolic toxicities of antiretroviral therapy.
AUTHOR: Herman J S; Easterbrook P J

CORPORATE SOURCE: Department of HIV/GUM, The Guy's, King's and St Thomas'
School of Medicine, King's College Hospital, Cutcombe Road,
London SE5 9RS, UK.
SOURCE: INTERNATIONAL JOURNAL OF STD AND AIDS, (2001 Sep) 12 (9) :
555-62; quiz 563-4. Ref: 71
Journal code: 9007917. ISSN: 0956-4624.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010823
Last Updated on STN: 20011015
Entered Medline: 20011011

AB Since the adoption of highly active antiretroviral therapy (HAART) in the mid-1990s, certain metabolic toxicities have been increasingly recognized. These include a fat redistribution syndrome (lipohypertrophy, lipoatrophy), hyperlipidaemia, altered glucose metabolism and insulin resistance, mitochondrial toxicity (presenting as anaemia, myopathy, pancreatitis, neuropathy, hepatic steatosis and lactic acidosis), and bone density abnormalities (osteoporosis and osteonecrosis). Metabolic **complications** are principally reported with protease inhibitors and nucleoside reverse transcriptase inhibitors, but may be seen with all classes of antiretroviral therapy. In this **review**, we summarize the epidemiology, pathogenesis and management of these various toxicities.

L9 ANSWER 2 OF 44 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:199765 CAPLUS
DOCUMENT NUMBER: 137:107007
TITLE: Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency
AUTHOR(S): Merke, Deborah P.; Bornstein, Stefan R.; Avila, Nilo A.; Chrousos, George P.
CORPORATE SOURCE: Warren Grant Magnuson Clin. Cent., Natl. Inst. Child Health Human Dev., Natl. Inst. Health, Bethesda, MD, USA
SOURCE: Annals of Internal Medicine (2002), 136(4), 320-334
CODEN: AIMEAS; ISSN: 0003-4819
PUBLISHER: American College of Physicians-American Society of Internal Medicine
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A **review**. Congenital adrenal hyperplasia describes a group of inherited autosomal recessive disorders characterized by an enzymic defect in cortisol biosynthesis, compensatory increases in corticotropin secretion, and adrenocortical hyperplasia. 21-Hydroxylase deficiency is responsible for more than 95% of cases and is one of the most common known autosomal recessive disorders. The classic or severe type presents in the newborn period or early childhood with virilization and adrenal insufficiency, with or without salt loss; the mild or nonclassic form presents in late childhood or early adulthood with mild hyperandrogenism and is an important cause of masculinization and infertility in women. This wide range of phenotypic expression is mostly explained by genetic variation, although genotype-phenotype discrepancies were described. Reproductive, **metabolic**, and other comorbid conditions, including risk for tumors, are currently under investigation in both forms of the **disease**. A high proportion of patients with adrenal incidentalomas may be homozygous or heterozygous for 21-hydroxylase deficiency. Women with congenital adrenal hyperplasia often develop the polycystic ovary syndrome. Ectopic adrenal rest tissue is often found in the testes of men with congenital adrenal hyperplasia; characteristic clin. and radiol. findings help differentiate this tissue from other tumors. Levels of corticotropin-releasing hormone are elevated in patients with depression and anxiety and are expected to be elevated in patients with congenital adrenal hyperplasia; it is unknown whether patients with 21-hydroxylase deficiency have an increased incidence of these psychiatric disorders. Abnormalities in both the structure and function of the adrenal medulla were shown in patients with classic congenital adrenal hyperplasia, and the degree of adrenomedullary impairment may be a biomarker of disease severity. The 21-hydroxylase-deficient mouse has provided a useful model with which to examine disease mechanisms and test new therapeutic interventions in classic disease, including gene therapy. Treatment of this condition is intended to reduce excessive corticotropin secretion and replace both glucocorticoids and mineralocorticoids. However, clin. management is often **complicated** by inadequately treated hyperandrogenism, latrogenic hypercortisolism, or both. New treatment approaches currently under investigation include **combination therapy** to block androgen action and inhibit estrogen prodn., and bilateral adrenalectomy in the most severely affected patients. Other approaches, which are in a preclin. stage of investigation, include treatment with a corticotropin-releasing hormone antagonist and gene therapy.

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NEUROCHEMICAL RESEARCH, (1999 Apr) 24 (4) 601-15

Drugs (2002), 62(5), 733-742

Medicine (Baltimore, MD, United States) (2002), 81(1), 69-8

Journal of Inherited Metabolic Disease (2001), 24(2), 275-290

Klinische Pharmakologie (1994), 11(Drug Interaction Studies during Drug Development), 38-42

Thank you.

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LEADING ARTICLE

Drugs 2002; 62 (5): 733-742
0012-6667/02/0005-0733/$30.00/0

New Prospects for the Treatment of Lysosomal Storage Diseases

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Abstract

Although individually rare, lysosomal storage disorders constitute a significant burden on society. To date, enzyme replacement therapy (ERT) has been the most successful therapeutic approach for lysosomal storage disorders.

ERT reverses systemic manifestations of Gaucher disease but does not effectively treat the neurological complications. Recently, ERT produced a reduction of severe neuropathic pain, stabilisation of renal disease, and improved vascular function and structure in short-term, placebo-controlled trials in patients with Fabry's disease. Long-term studies are necessary to evaluate the full potential of ERT in this disease. In patients with Pompe disease, a fatal cardiac and skeletal muscle disorder, ERT improved cardiac function and structure, and increased overall muscle strength. It has already increased survival in a small number of affected infants. ERT also decreased liver and spleen size, joint mobility and quality of life in patients with mucopolysaccharidosis type I, but when the therapeutic protein is administered intravenously, it is unlikely to modify the neurological outcome in this or in other similar disorders.

Bone marrow transplantation continues to be effective in Gaucher disease, in some forms of mucopolysaccharidosis and in mild forms of Krabbé disease, but it has high morbidity and mortality that limits its use in lysosomal storage disorders. Drugs that slow the rate of formation of accumulating glycolipids are being developed and one of them, OGT-918 (N-butyldeoxynojirimycin), is showing promise in patients with Gaucher disease. Gene therapy for lysosomal storage disorders holds promise as a replacement for the other therapies described here but requires much more development before clinical efficacy trials.

Lysosomal storage disorders are a group of recessive genetic disorders resulting from deficiencies of acid hydrolases.[1] Although individually rare, lysosomal storage disorders have a prevalence of 1 per 7700 live births and therefore constitute a significant burden on society.[2] A number of recent advances have occurred in the treatment of these enzymopathies. In this review we describe each treatment modality separately in order to emphasise and compare the characteristics, difficulties and challenges of the therapeutic approach in each disorder. We discuss enzyme replacement therapy (ERT), the most useful therapeutic approach thus far, in greater detail followed by bone marrow transplantation (BMT), substrate deprivation and an update on the latest efforts in gene therapy.

1. Enzym Replacem nt Th rapy

1.1 Gaucher Disease

Gaucher disease is the most prevalent lysosomal storage disorder.[3] It is an autosomal recessive condition caused by a deficiency of glucocerebrosidase. This defect leads to a multisystem disorder consisting of progressive visceral enlargement and gradual replacement of the haematopoietic system with lipid-laden macrophages.[4] Symptomatic anaemia, thrombocytopenia, hepatosplenomegaly and skeletal damage occur in most patients.

ERT with macrophage-targeted glucocerebrosidase has matured into the standard therapy for the non-neuronopathic form (type I) of the disease.[5,6] More than 3200 patients are currently receiving imiglucerase. ERT reverses the anaemia, thrombocytopenia and organomegaly in these patients and clears storage from bone marrow. When initiated early in life, ERT results in normal bone structure and skeletal development even in patients with underlying aggressive disease.[7] The frequency of painful bone crises and bone fractures appear to have markedly decreased in adult patients with type I Gaucher disease,[8] although this has not been formally demonstrated in comparative treatment trials.

ERT is also effective systemically in chronic neuronopathic Gaucher disease, especially when initiated at a young age.[7] The therapy has dramatically modified the clinical outcome of these patients who, prior to ERT, succumbed to pulmonary, hepatic and neurologic complications as teenagers.[9]

Intravenous infusions of glucocerebrosidase every 2 weeks have a very good safety profile with no known overdosing complications. Outside of very rare immune-mediated infusion reactions or development of neutralising antibody, the main drawback appeared to be the high cost of this treatment. Therefore, in the first 4 to 5 years after introduction, there was much debate about the dose required. By now it has become clear that the ERT regimen should be individualised.[6] Patients with relatively mild disease such as those of Ashkenazi Jewish ancestry with the *N370S* mutations may require imiglucerase in the range of 15 to 30 IU/kg bodyweight every 2 weeks. On the other hand, patients with the chronic neuronopathic form (type III), who often have aggressive disease, require 60 to 120 IU/kg bodyweight every 2 weeks for optimal benefit.[10] In patients with type I Gaucher disease, rapid beneficial response is often obtained with an initial dose of 60 IU/kg for 6 to 12 months, and it is then reduced to lower maintenance doses.[6]

A number of limitations and questions remain with regard to the treatment of Gaucher disease.

ERT is ineffective in patients with the acute neuronopathic form (type II).[11] In these patients, enzyme therapy has a moderate effect on the haematological abnormalities but does not modify the fatal neurological outcome. Outside of anecdotal reports, ERT has no effect on the neurological manifestations of chronic neuronopathic Gaucher, although contrary to its effects in type II disease, this therapy is life-saving in this form of the disease.[7]

More than half of the children with type III Gaucher develop interstitial lung disease observed on chest radiographs or computed tomography (CT) scans that so far has not led to clinically overt respiratory difficulties, but the long-term outcome is not known. Pulmonary hypertension was observed in type I patients and can be detected by echography of physiological tricuspid regurgitation.[12] The occurrence of this complication before or even after ERT initiation has fuelled a debate about the causative role of the therapy.[13,14] It is possible in the rare patient with pre-existing subclinical pulmonary hypertension and severe liver involvement that ERT causes closure of 'protective' pulmonary arterio-venous shunts, which leads to evident pulmonary hypertension. However, this complication is less common in treated patients compared with those that are untreated and it usually tends to resolve with ERT. Nevertheless, it is currently recommended that all patients with Gaucher disease be screened for the presence of pulmonary hypertension and subsequently that

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pulmonary pressure be followed during at least the first year on therapy.[15]

Initial reports on the ability of bisphosphonates to reduce risk of bone fractures in patients with Gaucher disease [16] has led a placebo-controlled trial comparing ERT alone with ERT plus bisphosphonates. In that study the bone density remained unchanged on ERT alone while it significantly increased when ERT and alendronate therapy were combined.[17]

Patients with Gaucher disease have a higher incidence of haematological malignancies, yet it is not known whether ERT will reduce this risk over time.

1.2 Fabry Disease

Fabry disease is an X-linked disorder resulting from mutations of the α-galactosidase A gene.[18] The defect results in very low activity of the lysosomal enzyme α-galactosidase A with impaired metabolism of terminal α-D-galactosyl moieties.[19] Systemic glycosphingolipid accumulation occurs in vascular endothelial and smooth muscle cells, myocardium, renal epithelium, the dorsal root and autonomic ganglia in the peripheral nervous system and neurons in the central nervous system (CNS).[20] Patients develop a painful small fibre neuropathy and hypohydrosis followed by progressive renal, cardiac and cerebrovascular deterioration. Median survival of these patients is 53 years.[21]

It should be stressed that unlike Gaucher disease, Fabry disease is a multisystem and multiorgan disorder associated with progressive damage and functional loss.[20] In this respect it resembles processes such as atherosclerosis rather than a classic storage disorder, with a reduced likelihood of functional reversal once the primary defect is reversed. The renal insufficiency is the end result of decades of progressive nephron loss, and the strokes illustrate destructive processes that are unlikely to reverse with specific therapy. However, such therapy may be helpful to prevent further deterioration, and can theoretically rescue dysfunctional cells before they reach the 'point of no return'. On the other hand, sweat glands are present in normal numbers in the skin and, therefore, their dysfunction is likely to be reversed with specific therapy.

Two groups have been developing ERT for Fabry disease. Our group has been using α-galactosidase A produced in a genetically engineered continuous human cell line.[22,23] In our initial study single intravenous infusions of escalating doses of α-galactosidase A over the range of 0.007 to 0.11 mg/kg bodyweight were administered to five groups of two patients each.[22] The initial half-life of the enzyme in the plasma was 20 minutes and the terminal half-life (obtained from the non-compartmental model) ranged from 42 to 117 minutes with an average of 83 minutes. Similar values were obtained using a 3-compartment model.[22] Approximately 50% of the enzyme was found in the liver where its half-life was 2 days. Immunochemical staining revealed that the enzyme was present in Kupffer and sinusoidal endothelial cells, and to a lesser extent, in hepatocytes. Following infusion of α-galactosidase A, there was a mean decrease of 31% of accumulated globotriaosylceramide (Gb_3) in the liver ($p < 0.05$). The reduction, however, did not appear to be proportional to the quantity of enzyme infused. Urinary Gb_3 is greatly elevated in patients with Fabry disease. Following the single injection of α-galactosidase A, the mean decrease of Gb_3 in the urine sediment 28 days post-infusion was 38% ($p < 0.01$). These observations indicated that the injected enzyme reached the renal tubular cells and reduced the amount of accumulated Gb_3 in them.

Following the initial trial, we studied the clinical efficacy and safety of ERT in a 6-month, double-blind, placebo-controlled trial using 0.2 mg/kg bodyweight or approximately 10 IU/kg.[23] For practical reasons and on the basis of the success of ERT in Gaucher disease, we opted to administer enzyme infusions every 2 weeks. We found that patients receiving ERT had a significant reduction in neuropathic pain (primary outcome measure) and a significant improvement in pain-related quality of life.[23] Patients receiving enzyme treat-

ment had a significant improvement in renal pathology, with about a 16% deterioration in these parameters in the placebo group.[23] There was also a significant reduction in lysosomal inclusions in kidney vascular endothelial cells in the ERT group compared with placebo. These structural changes were reflected in stable or improved renal function with ERT and deterioration in inulin clearance with placebo, although the difference did not reach statistical significance.

Using [^{15}O] H_2O positron emission tomography (PET), we found abnormally elevated resting regional cerebral blood flow and acetazolamide-induced cerebrovascular hyper-reactivity in 26 patients with Fabry disease. These abnormalities were reversed with ERT.[24] Because of the known involvement of nitric oxide (NO) in the control of resting cerebral blood flow, we examined skin biopsies and archived brain immunohistochemically for nitrotyrosine, and found enhanced nitrotyrosine staining in dermal and cerebral blood vessels. These findings suggest a chronic alteration of the NO pathway in Fabry disease with protein nitration. The increased staining for nitrotyrosine in dermal blood vessels was reversed with ERT.[24] Patients receiving enzyme therapy had a significant increase in bodyweight with reduction in Gb_3 in plasma and urinary sediment, indicating a substantial metabolic improvement with ERT.[23]

After the controlled trial, patients who had received placebo then received enzyme infusions and experienced the same benefits as the original ERT group. ERT was very well tolerated. Mild infusion reactions occurred, but they were easily controlled and they tended to diminish and even disappear with repeated infusions. Nine of 12 patients developed low immunoglobulin (Ig)G anti-α-galactosidase A titre by the immunoprecipitation assay. The titre tended to decrease with repeated infusions and did not correlate with the presence of infusion reactions. Currently, α-galactosidase A is administered as home therapy to patients without any difficulties.

Eng et al. used α-galactosidase A prepared in Chinese hamster ovary cells.[25] They first performed a dose-finding study in which 15 patients with Fabry disease received five intravenous doses of recombinant α-galactosidase A administered over 120 minutes.[25] The regimens were 0.3 mg/kg of bodyweight every 14 days (biweekly), 1.0 mg/kg biweekly, 3.0 mg/kg biweekly, 1.0 mg/kg every 48 hours, and 3.0 mg/kg every 48 hours. Elimination half-life was similar that seen in our study. Plasma, liver and renal Gb_3 were reduced in a dose-dependent manner. Histological clearance of lysosomal inclusions was observed in liver, heart and skin. Clearance of Gb_3 was seen mostly in vascular endothelial cells. IgG antibodies developed in 54% of the patients, one of whom had infusion reactions preventing re-infusion of the enzyme preparation. No neutralising antibodies were detected.[25]

Following on from these results, the same group conducted a 20-week (11 infusions), placebo-controlled, multicenter trial in 58 relatively young and healthy patients with Fabry disease. On the basis of the first trial, they chose the dose of 1 mg/kg bodyweight every 2 weeks administered over 3 to 5 hours.[26] The primary outcome measure was the number of patients with no microvascular endothelial deposits of Gb_3 in renal biopsies in methylene blue-azure II-stained sections.[26] This endpoint was chosen on the basis of the assumption that renal dysfunction in Fabry disease is entirely related to vascular endothelial abnormalities. Patients with the so-called cardiac variant, who do not develop renal insufficiency, rarely have vascular endothelial deposits; therefore, reversal of this abnormality should lead to preservation of renal function.[27] The primary efficacy endpoint of the double-blind study required more than 50% of the renal interstitial capillaries in each specimen to have a score of 0, less than 5% to have a score of 1 or greater, and the remainder to be designated as having trace evidence of microvascular endothelial deposits of Gb_3 after week 20.[26] The authors similarly examined Gb_3 deposits in skin and in heart biopsies.

At the end of the study, 20 of the 29 patients in the recombinant α-galactosidase A group (69%) had no microvascular endothelial deposits of Gb_3 after 20 weeks, compared with none of the 29 pa-

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tients in the placebo group ($p < 0.001$). Similar results were obtained in skin and heart biopsies. Pain and quality of life were not significantly different between ERT and placebo groups. In the following nonblind 6-month study, patients who crossed over to receive enzyme therapy had a reduction in endothelial deposits comparable to that of the original ERT group.[26]

With this α-galactosidase A product, patients commonly experienced mild to moderate infusion reactions that required prolongation of infusion time and anti-inflammatories. The therapy had to be discontinued in one patient because of positive skin test to infused enzyme. Eighty-eight percent of patients seroconverted to anti-α-galactosidase A IgG with evidence of tolerisation with repeated infusions. The authors have not commented on a possible relationship between the presence of an antibody titre and the occurrence of infusion reactions.[26]

At this stage, it is difficult to directly compare the clinical efficacy of these two α-galactosidase A preparations. The doses administered cannot be directly compared since the specific activity of the Chinese hamster ovary cell product has not yet been published, and neither was the complete and detailed glycosylation pattern of either product revealed. It is likely that both forms of α-galactosidase A enter cells mostly via the mannose-6-phosphate receptor. Using the human cell line derived enzyme we demonstrated, in addition to a reduction in storage in renal vascular endothelial cells, clear clinical benefit. At this point, one cannot determine whether the different results are due to a qualitative difference of the α-galactosidase A made in human cells from the one made in Chinese hamster ovary cells, or to a different clinical trial design and the patient population studied, or a combination of both factors.

Long-term studies are required to evaluate the effect of ERT on critical clinical endpoints such as time to end-stage renal disease, cardiac insufficiency, stroke, quality of life and survival rate.

1.3 Pompe Disease

Pompe disease is a fatal autosomal disorder caused by lysosomal α-glucosidase deficiency.[2] Infants develop hypotonia as a result of central and peripheral nervous system involvement. Lysosomal glycogen storage in cardiac and skeletal muscle represent the most readily accessible targets for enzymatic correction. Here again at least two groups have been developing α-glucosidase ERT.

The first group produced recombinant α-glucosidase in rabbit milk and administered 15 to 40 mg/kg bodyweight to four infants with Pompe disease. Cardiac size and function improved, and enzyme activity normalised in muscle on the highest enzyme dose.[28,29] Infusion reactions related to IgG antibody included fever, rash and hypoxia, but slowing the infusion rate controlled them.

A second group used α-glucosidase made in Chinese hamster ovary cells and also administered it in another nonblind trial to three patients for up to 17 months.[30] ERT was administered twice a week at a dose of 5 mg/kg (specific activity 1.8×10^5 nmol/h/mg protein), a much lower dose than the rabbit milk preparation. Infusion reactions were rare but complement-activating IgG anti-α-glucosidase hampered treatment in two of the three patients. Cardiac size and function improved, and all patients achieved the primary outcome measure that was living past 1 year of age without developing cardiac failure. Improvement in motor strength was also observed. It remains to be seen how these patients and others will do over the long term since the CNS is also involved with dysfunction of lower motor neurons in the cranial nerves and spinal cord.

1.4 Mucopolysaccharidoses

The success of ERT in Gaucher disease has led to interest in using a similar approach for the mucopolysaccharidoses. These disorders have in common with Gaucher disease hepatosplenomegaly, marked skeletal abnormalities and CNS disease.

Thus far, only one nonblind trial has been published of the use of ERT in 10 patients with mucopolysaccharidosis type I, which is caused by a deficiency of the alpha-L-iduronidase.[31] Patients with this metabolic defect have varying degrees of progressive developmental delay, corneal clouding, airway obstruction, cardiac disease, hepatosplenomegaly and severe joint restriction. Most die by the age of 10 years. The enzyme was made in Chinese hamster ovary cells. Patients received 100 IU/kg every week administered over 3 hours, or up to 6 hours if hypersensitivity reactions occurred, for 52 weeks.

Hepatosplenomegaly decreased by a mean of 25%. Mean urinary glycosaminoglycan excretion declined by 64% by week 52. Growth rate as measured by height and weight, in the six prepubertal patients, increased by a mean of 85 and 131%, respectively. The mean maximal range of motion of shoulder flexion and elbow extension increased significantly.[31] The number of episodes of apnoea and hypopnea during sleep decreased 61%. New York Heart Association (NYHA) functional class improved by one or two in all patients and all patients reported increased physical endurance. All patients developed antibodies to Chinese hamster ovary proteins and four patients developed anti-α-L-iduronidase antibody of the IgG type, some with evidence of complement activation. Five patients had urticaria or facial swelling during infusions.[31]

This nonblind trial suggested that recombinant human alpha-L-iduronidase may be useful for a number of non-CNS manifestations of mucopolysaccharidosis type I. A controlled study has been initiated to confirm these findings.

There are also plans to evaluate the use of ERT in patients with Hunter disease (mucopolysaccharidosis type II), Maroteaux-Lamy disease (mucopolysaccharidosis type VI) and even in Sanfilippo syndrome type B (MPS III B) that has been explored in animal models.[32] Although systemic improvement is likely to be possible with ERT in these diseases, no effect on their neurological manifestations is anticipated. An exception may be mucopolysaccharidosis type VII that is very rare. On the basis of pre-clinical studies, only minute amounts of enzyme may be required to obtain neurological benefit.[33]

2. Bone Marrow Transplantation

Hundreds of patients with lysosomal disorders have been treated with BMT over the years.[34] Because of the selective involvement of bone marrow-derived cells, type I Gaucher disease remains the prototype of this therapy since successful engraftment cures the disease. On the other hand, there is little evidence that BMT has an effect on the neurological abnormalities in chronic neuronopathic Gaucher disease.[35]

BMT has more limited effect in the other disorders. In mucopolysaccharidosis type VI (Maroteaux-Lamy disease), BMT stabilised or slightly improved the general condition, cardiomyopathy and facial features, but skeletal benefit was limited.[36] The authors of this study concluded that BMT can prolong life and improve its quality in well-chosen patients. Benefit was seen also in cell therapy using cord blood from an affected matched brother with Maroteaux-Lamy disease with improvement of quality of life, facial features and joint mobility.[37] Similar effects of BMT may be seen in mucopolysaccharidosis type I or II, but again, no effect was seen on the neurological progression.[34,38] BMT improved nerve conduction and head magnetic resonance imaging (MRI) abnormalities in patients with relatively mild Krabbé disease (globoid cell leukodystrophy), and stabilised the neurologic deficit.[39] Mild forms of metachromatic leukodystrophy may also benefit from BMT.[34]

3. Substrate Deprivation

The principle behind this approach is reduction in storage material by inhibiting synthetic enzymes using small molecules. In theory, this approach should work best when some residual enzyme activity is present such as in patients with Gaucher disease.[40] These small molecules are more likely to cross the blood-brain barrier than large proteins making this approach attractive for the treatment

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At present, the most advanced drug is OGT-918 (N-butyldeoxynojirimycin; NB-DNJ).[42] OGT-918 is an N-alkylated imino sugar that specifically inhibits biosynthesis of all glucosylceramide-based glycosphingolipids by reversibly inhibiting glucosylceramide synthase. While a complete inhibition of this enzyme is not compatible with normal embryonic development,[43] partial inhibition does not seem to have deleterious effects.[44] In animal models, OGT-918 improved survival of Tay-Sach and Sandhoff mouse models,[45] and this drug enhances the beneficial effects of BMT.[46]

OGT-918 was recently evaluated in a nonblind study in patients with type 1 Gaucher disease.[42,47] It decreased liver and spleen size, increased haemoglobin and platelet counts, and reduced blood glucosylceramide and chitotriosidase levels.[42] The dosage used varied from 100 to 300 mg/day. Other trials that were designed to compare the effect of OGT-918 alone or together with imiglucerase in patients with Gaucher disease, and a phase I study in patients with Fabry disease, have been completed. These results have not yet been reported. Diarrhoea as a result of inhibition of intestinal disaccharidases has been the main adverse effect of OGT-918. Some patients developed reversible paresthesias or tremor, and two cases of peripheral neuropathy were reported.[42,47] Their relationship to the drug is unclear.

Other inhibitors of glucosylceramide synthase are being developed but they are all in pre-clinical stage of development.[48-50] The ultimate usefulness of this treatment approach cannot be assessed at this early stage.

4. Use of Small Molecules

It has been recently found that the addition of galactose or certain reversible competitive inhibitors of α-galactosidase A, such as 1-deoxygalactonojirimycin, increases the activity of the enzyme.[51] A patient with Fabry disease and severe cardiomyopathy was treated with intravenous infusions of galactose at 1 g/kg bodyweight, administered over a 4-hour period every other day for over 2 years.[52] With this treatment there was a marked clinical improvement in cardiac function, endocardial pathology, and quality of life of the patient. Increased enzyme activity in peripheral blood white cells and cardiac tissue occurred with galactose infusion, but the authors did not indicate the time-relationship of the sampling to the sugar infusion.[52]

This paper is important as a proof of principle but this therapy is not very practical, and by virtue of the rarity of the susceptible α-galactosidase A mutations, is limited to a relatively small proportion of patients with Fabry disease. In addition, the authors have not reported the ultimate outcome of this patient. It is possible that other agents that are more easily administered can be developed in a number of disorders that result from partial deficiency of enzymes that can be activated. A novel pharmacological approach is the use of small molecules that can directly disrupt chemical linkages of substrates instead of the deficient lysosomal enzyme.[53]

5. Gene Therapy

Gene transfer has not yet realised its potential and development has lagged behind the rapid identification of the causative genes of the various lysosomal storage disorders. Enzyme deficiencies such as lysosomal disorders are good candidates for gene therapy. The ideal gene therapy should of course attempt to genetically correct every cell in the body. However, because of lack of adequate modes to deliver gene therapy in a widespread manner, this goal is unlikely to be achieved in the near future.

Since most of these disorders result from enzymatic deficiencies, the approach has been to overexpress the normal gene by transferring it to a patient's cells either directly *in-vivo* (for example intravenously to liver tissue or brain)[54,55] or *ex-vivo* to haematopoietic stem cells or fibroblasts followed by autotransplantation.[56] Some of these disorders can be effectively treated with only a small increase in enzymatic levels above the defi-

cient state. The over-expressing genes serve as a biological enzyme pump that delivers normal enzyme via the general circulation.[57] Therefore, this approach to gene therapy is likely to have similar advantages and limitations, as does intravenous ERT.

Other potential limitations include the development of immune response to the therapeutic enzyme or to other components of the vector delivery system. These complications cannot usually be treated by removal of the offending protein. In addition, the delivery of corrective genetic material to the brain remains a difficult but necessary goal, although recent studies in animal models seem promising.[55,58]

Pre-clinical studies have been reported in Fabry disease[54] and in the mucopolysaccharidoses,[56,59] while clinical phase I studies have been performed in patients with type 1 Gaucher disease.[60,61] A phase I/II study involving retroviral-mediated transfer of iduronate-2-sulfatase gene into lymphocytes of patients with mucopolysaccharidosis II (mild Hunter Syndrome) is currently recruiting. It is sponsored by the National Institute of Child Health and Human Development (NICHD), University of Minnesota, USA.[62]

6. Conclusions

In the past few years there has been a significant advance in the treatment of lysosomal storage disorders. ERT turned out to be perfectly suited for type 1 Gaucher disease and this success made it the most prevalent form of specific therapy currently being developed. It remains to be seen to what extent this approach will benefit patients with other lysosomal disorders.

Other methods are clearly required to prevent or reverse the abnormalities in the brain and other elusive tissue compartments. These necessary advances should come from improved delivery methods of proteins[63,64] and genes,[65] and from the elucidation of the pathogenesis of these disorders.

Methods for evaluating the responses to the various forms of therapy require particular attention. Treatment trials should use clinically relevant outcome measures rather than biological markers. Ultimately, it is necessary to demonstrate that the remedy under study significantly improves the natural history of critical medical events and patient quality of life.

Acknowledgements

Funding/Support: The Intramural Program of the National Institutes of Health supported the study. Dr Brady has a Cooperative Research and Development Agreement (CRADA) with Transkaryotic Therapies Inc, Cambridge, Mass and is an approved consultant for Genzyme Corporation, Cambridge, Mass.

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Thank you.

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Gene therapy for the lysosomal storage disorders

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Current Opinion in Molecular Therapeutics (2002) 4(4):349-358
 ISSN 1464-8431

The lysosomal storage disorders (LSD) are monogenic inborn errors of metabolism with heterogeneous pathophysiology and clinical manifestations. In recent decades, these disorders have been models for the development of molecular and cellular therapies for inherited metabolic diseases. Studies in preclinical in vitro systems and animal models have established proof-of-concept for the development of bone marrow transplantation (BMT) and enzyme-replacement therapy (ERT) as therapeutic options for several LSDs. BMT is limited by poor donor availability and high morbidity and mortality, and although ERT is a good treatment, it is not a life-long cure. Its high cost remains an impediment for developing countries. While substrate synthesis inhibition therapy is an important idea, its clinical use is far from certain. The neuropathology present in many LSDs has responded poorly to BMT or ERT, which makes gene therapy an attractive therapeutic alternative. Oncoretroviral vectors, and more recently adeno-associated and lentiviral vectors have been tested with some success. This review summarizes the main gene therapy strategies which have been employed or are under development for both non-neurological and neuronopathic LSDs. Some of the in vitro and in vivo preclinical studies presented herein have provided the rationale for gene therapy clinical trials for Gaucher disease Type 1.

Keywords Animal models, BMT, enzyme replacement, gene therapy, gene transfer, lysosomal storage disorders

Introduction

The lysosomal storage diseases (LSDs) are a group of inherited disorders for which gene therapy offers a potential cure. The genes encoding the lysosomal enzymes have been cloned and characterized, and the gene products have been shown to be therapeutic. As several laboratory studies and clinical experience have demonstrated, enzyme-replacement therapy (ERT) reverses the visceral complications of lysosomal storage in several diseases, including Gaucher disease [1,2], Fabry disease [3,4••,5], Pompe disease [6,7••], Hurler-Scheie disease or mucopolysaccharidosis Type I (MPS I) [8] and Hunter disease (MPS II) [9]. Based on the evidence gathered by allogeneic bone marrow transplant (BMT), it has been established that engrafted hematopoietic cells reverse the signs and symptoms in some of the LSDs and may therefore be appropriate targets for *in vivo* or *ex vivo* gene transfer [10]. Correction of these cells should allow repopulation of the recipient hematopoietic and lymphoid cells with normal donor-derived cells, thereby providing a self-renewing source of the deficient gene product, which is to be produced by cells of the monocyte-macrophage lineage. Donor cells may also release the deficient gene product and correct neighboring or distant cells that should be of benefit through the process of receptor-mediated endocytosis [11]. Efficient gene delivery to a cell involved in the pathobiology of the disease is a primary concern for the development of gene therapy strategies for the LSDs. Such genetic correction is likely to be efficacious, stable and safe. Furthermore, for cells such as hematopoietic stem cells (HSCs), appropriate homing of the corrected cells to the right compartment is a further challenge after successful gene transfer and expression has been achieved for some disorders. At present, viral-mediated gene transfer is the most promising approach, and provides the most efficient gene transfer. Another challenge for the development of gene transfer as a therapeutic tool is the presence of central nervous system (CNS) involvement in most of the LSDs. Strategies must be aimed at overcoming the limitations imposed by the blood-brain barrier (BBB), as well as the antenatal injury resulting as a consequence of the genetic disease, and will probably require the improvement of both *in utero* and intracranial gene transfer.

For the most part, the pathophysiology of the lysosomal storage disorders is caused by an enzymatic deficiency that results in the storage of undegraded macromolecules. However, there are other types of lysosomal storage diseases caused by defects in transporter proteins, with the result that certain compounds are unable to leave the lysosome; this is the case for cystinosis and sialic acid storage disorders [12]. In the case of Niemann-Pick disease type C, cholesterol lysosomal storage is secondary to a defect of cholesterol recycling [13]. This review emphasizes the gene transfer approaches for the treatment of inherited enzymopathies that produce lysosomal storage diseases.

Murine models of lysosomal diseases

Animal models of LSDs are helpful, particularly when the animal phenotype has a resemblance to the human disease. One of the most extensively studied models is the naturally occurring MPS VII (*gus* $^{mps/mps}$). These mice have been found to have morphological, genetic and biochemical characteristics that closely mimic those of human MPS VII [14], including facial dysmorphism, growth retardation, deafness, behavioral deficits and shortened life-span. A recently produced transgenic mouse expressing the human β-glucuronidase (GUSB) cDNA with an amino acid substitution at the active site (nucleotide (E540A)) was bred onto the MPS VII (*gus* $^{mps/mps}$) background [15]. Interestingly, this new transgenic mouse tolerates immune challenge with human GUSB and may be useful for preclinical trials to evaluate the effectiveness of ERT and/or gene therapy approaches. Another example of a naturally occurring murine model is the *twitcher* mutant for Krabbe disease [16], which is useful for the development of therapies for the sphingolipidoses. A summary of the current animal models for LSDs is provided in Table 1.

Table 1. Animal models for the lysosomal storage disorders.

Disease	Target	Vector	Response
Gaucher disease	HSC Liver Myoblasts	RV AAV RV	Long-term expression in mice Correction of lipid storage in mouse model
MPS VII	HSC Liver Brain	RV Ad, AAV AAV LentiVirus, Ad	Correction of liver, bone not brain Correction of liver, bone not brain Correction of biochemistry and pathology
Fabry disease	HSC Liver	RV Ad, AAV	Correction of lipid storage, pathology in mice Correction of lipid storage, pathology in mice
Metachromatic leukodystrophy	HSC	RV LentiVirus	Correction of biochemistry in extraneural tissue Correction of brain pathology
Niemann-Pick disease	HSC	RV	Correction of biochemistry and increased survival
MPS I	HSC	RV	Biochemical effects, immune rejection
MPS II	HSC	RV	Enzyme expression, biochemical effect
MPS III B	Fibroblasts Brain	AAV	Enzyme expression in brain, biochemical effect Bystander effect in brain
MPS VI	Tissue culture	RV	Enzyme expression, biochemical effect

Ad adenovirus; **AAV** adeno-associated virus vector; **HSC** hematopoietic stem cell; **MPS** mucopolysaccharidosis; **RV** retrovirus.

For diseases that have no natural model, gene targeting in pluripotent mouse embryonic stem cells has allowed the development of murine models for most human LSDs. Such developments include the knockout mouse model for Fabry disease, which displays a complete lack of α-galactosidase A (AGA) activity, with accumulation of the substrate in the liver, kidneys and cultured fibroblasts [17], and the Pompe disease murine model [18]. These models have been useful in developing both enzyme and gene therapy strategies, although they do not exactly mimic the observed phenotype in humans. These phenotypic differences from those observed in humans are the consequence of alternative metabolic pathways of lysosomal metabolism in rodents. An example is the transgenic glucocerebrosidase-deficient mouse model generated for Gaucher disease studies [19]. This model and other targeted mutations exhibited a profound, severe phenotype with prenatal or perinatal lethality that precluded its use in most studies of gene transfer and cell transplantation. Similarly, an arylsulfatase (ASA)-deficient mouse generated for metachromatic leukodystrophy (MLD) showed lipid storage patterns in fibroblasts, CNS oligodendrocytes and peripheral nerves similar to the human MLD phenotype, but without gross defects of the white matter in the first two years of life [20]. Mice with a targeted knockout of the gene encoding β-hexosaminidase A (whose deficiency is responsible for Tay-Sachs disease) [21] are another example of the phenotypic differences between the mouse model and the corresponding disease in humans.

Another useful animal model has been generated for MPS III B (Sanfilipo syndrome), an LSD caused by a deficiency of α-*N*-acetylglucosaminidase. This knockout mouse model was generated by targeted disruption of exon 6 of the corresponding gene, and the mice remain healthy while young. Its biochemical phenotype is generally similar to the human disorder, but does not show increased urinary excretion of heparan sulfate or hyperactivity. In contrast to the affected humans, these mice are hypoactive [22]. Enzyme therapy has proven to be useful in reversing the visceral manifestations of the disease in this model, but has no effect on CNS involvement. In contrast, gene transfer approaches have produced satisfactory biochemical results [23].

Gene transfer for LSDs without prominent CNS involvement

Gaucher disease

Gaucher disease results from a deficiency of the lysosomal enzyme glucocerebrosidase (GC), which catalyses the cleavage of glucosyl-ceramide (glu-cer). The accumulation of glu-cer occurs primarily in the lysosomes of macrophages and affects mainly the reticuloendothelial system, thus impacting on multiple organ systems. Principal manifestations include hepatosplenomegaly, gradual replacement of BM and skeletal deterioration [24]. The most frequent variant of Gaucher disease, known as non-neuronopathic or type 1, does not present primary neuropathology. These characteristics of type 1 Gaucher disease make it a good candidate for the development of treatment strategies based on correcting enzyme deficiency in macrophages either by BMT, enzyme replacement or gene transfer. BMT in these patients showed a gradual reduction in organomegaly and presented histopathological evidence of clearance of Gaucher cells (glucosyl-ceramide-engorged macrophages), together with an improvement in the clinical status of the patients [25-27]. A commercial preparation of mannose-terminated GC, known as Cerezyme (Imiglucerase), is widely used for ERT Gaucher disease patients, and effectively reverses the visceral manifestations of the disease [1,24,28,29]. The success of BMT and reversal of clinical manifestations with ERT have shown that in the case of Gaucher disease, the enzymatic correction of only one cell type - the macrophage - results in effective therapy.

The effectiveness of BMT/ERT supports the rationale for permanent somatic cell gene therapy strategies aimed at transducing autologous BM stem cells; a less permanent strategy includes gene transfer to committed macrophage precursors, or peripheral blood monocytes. These may secrete the enzyme into the circulation for subsequent macrophage uptake. Success in long-term expression of the gene encoding GC in mouse hematopoietic cells has been demonstrated, as well as high-efficiency retroviral transduction of human CD34+ progenitor/stem cells obtained from patients with Gaucher disease [30-34].

Three clinical trials of HSC gene transfer and autologous transplantation without myeloablation for Type I Gaucher disease have been performed, and have been described in detail [35]. Only one showed substantial biochemical and clinical results, which were observed in just one out of four individuals.

Several improvements can be made to increase the efficiency of gene therapy of HSCs for Gaucher disease and other LSDs. Improvements can be made to the retroviral vectors, by using oncoretrovirus-derived constructs with a better expression profile [33], by employing more efficient promoters or different viral envelopes, or by using non-oncoretroviral vectors, such as the lentiviral (LV) vectors derived from HIV or other non-primate lentiviruses [34]. The main advantage of LV vectors over the oncoretroviral vectors is the capacity to infect non-dividing cells [36,37], which include human HSCs [34] and terminally differentiated neurons [36,38].

In vitro conditions that would increase the cycling of HSCs, such as GF stimulation and the use of cyclins, cyclin-dependent kinases and inhibitors, are being explored [33], as well as the optimal cytokine balance for enhancing the transduction of these cells. Another research focus is the BM microenvironment, which includes the stroma and molecules of the extracellular matrix, such as fibronectin, which has been shown to enhance HSC transduction. Another feasible improvement is the liposomal pretreatment of retroviral supernatants. This procedure synergistically enhances the effect of centrifugal enhancement on transduction efficiency of CD34+ cells [39]. This method is readily transferable to a clinical setting, and has been tested for large-scale clinical grade vector applications.

Another intriguing gene therapy strategy for Gaucher disease exists in the systemic delivery of GC by transplanted long-term expressing somatic cells that may secrete the enzyme in the circulation for subsequent macrophage uptake. Encouraging studies have shown that primary human myoblasts express and secrete GC *in vivo*; the secreted enzyme is then taken up by liver and BM macrophages [40,41].

Fabry disease

The encouraging results of ERT therapy for Fabry disease [4••] prompted several groups to explore the feasibility of gene therapy for patients with this condition. Fabry disease is caused by a deficiency in lysosomal hydrolase AGA, which leads to the accumulation of α-galactosyl-terminal lipids, such as globotriaosylceramide (Gb3). The main pathological alteration is represented by systemic vascular occlusion causing cardiovascular, cerebrovascular and renal disease. Unlike other LSDs, there is minor neuropathology, which mainly affects the dorsal root ganglia and the cells of the autonomic nervous system [42]. The principles learned from the development of ERT for Gaucher disease were applied to overexpress and secrete the lysosomal enzyme by gene-corrected cells for uptake by bystander cells [43,44]. This resulted in the development of gene transfer strategies, including viral and non-viral (plasmid-mediated) methodologies. The most impressive results have been obtained by Takenaka *et al*, who transplanted retrovirally transduced BM mononuclear cells in a murine model of Fabry disease [45•]. AGA-deficient BM mononuclear cells were retrovirally transduced to produce AGA, and then transplanted in the AGA-deficient mouse model. Long-term increased AGA activity and decreased Gb3 storage levels were observed in the hematopoietic organs, liver, heart, lung and kidney of sub-lethally and lethally irradiated primary and secondary recipient mice, despite a low percentage of marked cells. This lends experimental support to the hypothesis that even low levels of gene-corrected cells may be sufficient to achieve therapeutic efficacy in most LSDs; in Fabry heterozygotes, enzymatic levels of 10% of the normal value are compatible with a normal life-span and quality-of-life.

Another successful preclinical gene transfer strategy for Fabry disease has been developed by Jung *et al* using an adeno-associated virus (AAV)-derived vector [46]. AAV has been extensively used as a potential gene delivery vehicle [47,48]. In an *in vivo* gene delivery strategy, an AAV vector engineered to produce AGA under the EFI-A promoter was injected into the portal vein of AGA-deficient mice [46]. A single injection of the vector to the Fabry mouse liver was sufficient to restore AGA activity to levels necessary to correct widespread Gb3 storage, except in the kidney. The glycolipid storage levels remained at near-normal levels for up to 5 weeks post-injection, and persisted at levels that were 40 to 60% lower than the untreated Fabry animals for up to 6 months. This strategy of AAV vector delivery into a depot organ, such as the liver, proved to be non-toxic and did not elicit a significant immune response in mice, constituting a valuable gene therapy option for Fabry disease and other LSDs.

Pompe disease

Glycogen storage disease Type II (GSD-II), also known as Pompe disease, is a fatal genetic muscle disorder caused by a deficiency of acid α-glucosidase (GAA). This enzyme defect results in lysosomal glycogen storage in multiple tissues with cardiac and skeletal muscles being the most seriously affected [49]. The clinical severity of the disease and the absence of any effective therapy (although the results of the clinical trials of ERT for Pompe disease appear promising [6,7••]) prompted the development of specific *in vivo* and *ex vivo* gene therapy strategies. In *ex vivo* experiments, fibroblasts and myoblasts from GSD II patients were infectable by retroviral and adenoviral vectors encoding for GAA [50,51]. The transferred gene was efficiently expressed and the enzyme synthesized *de novo* reached lysosomes, where it digested glycogen. The transduced cells secreted GAA, which was endocytosed via the mannose-6-phosphate receptor into lysosomes of deficient cells and digested glycogen.

In vivo gene delivery approaches targeting the muscles of GSD II animal models have met with limited success. Efficient, systemic correction of the muscle disorder was obtained after intravenous injection of a modified adenovirus vector encoding human GAA in GSD II knockout mice [52•]. Hepatic transduction and secretion of high levels of the precursor GAA proenzyme into the plasma of treated animals was observed along with systemic distribution and uptake of the proenzyme into the skeletal and cardiac muscles of the mice. This produced

systemic decreases of the glycogen accumulations in a variety of muscle tissues. Despite this promising fact, other studies will be necessary to determine the long-term expression of the transgene and the potential immune responses in the animals. Moreover, it is not known if the improvement in the pathology resulted in improved muscle function in this study.

Gene transfer for LSDs with CNS involvement

Gene transfer and transplantation of BM cells

Gene transfer may be an even more important therapeutic alternative for LSDs with neurological involvement, because ERT and BMT have failed to improve the neuropathology of these disorders [53]. Gene transfer and transplantation of BM progenitors or mature blood cells such as macrophages may have a role in the treatment of storage diseases involving the CNS. This strategy may be efficacious through several possible mechanisms: (i) degradation of storage material may occur by genetically corrected enzyme-competent cells derived from the transplanted BM (sink effect), reducing the possibility of accumulation of the storage material in brain; (ii) secretion of the transgene product (enzyme) from BM-derived brain macrophages to neighboring cells (glia); and (iii) circulating enzyme may be endocytosed by macrophages destined for the CNS.

An LSD that may benefit from gene transfer and transplantation of BM cells is MLD, an autosomal recessive disorder of sulfatide metabolism that affects the formation and maintenance of myelin. Individuals with MLD are deficient in the activity of arylsulfatase A (ASA), a lysosomal enzyme that hydrolyzes galactosyl sulfatide (cerebroside sulfate). This single gene defect gives rise to lysosomal storage and accumulation of sulfatide in the white matter of CNS and peripheral nerves, and, to a lesser extent, in the visceral organs. It is believed that sulfatide-induced changes are responsible for the loss of myelination in the CNS and the range of ensuing neurological deficits that often result in death.

Transduction of murine BM with a vector carrying cDNA encoding arylsulfatase A (MFG-ASA) results in highly efficient gene transfer, as demonstrated by the presence of vector DNA in 90% of spleen colonies 12 days after BMT [54]. The vector sequence was detected in macrophage cultures obtained from transduced donor BM cells, and subsequently in the BM, spleen, lung, liver and brain of long-term reconstituted mice (4 months after BMT), indicating that BM-derived cells had migrated to and repopulated the viscera and the brain. Furthermore, the DNA signal was present in 100% of the spleen colonies of mice transplanted secondarily with BM from long-term reconstituted mice, indicating not only successful reconstitution, but also providing evidence of transduction of the pluripotent HSCs in the primary recipients. Enzyme activity in the brains of mice 4 months after BMT was unchanged as compared with non-transduced control samples, probably because of the small number of BM-derived cells that enter the brain. However, approximately two out of three spleen colonies obtained from secondarily transplanted mice expressed arylsulfatase A above control levels, indicating that the MFG vector was continuing to express the transgene. More recently, Matzner *et al* showed that 50% of ASA-deficient mice transplanted with BM cells retrovirally transduced with human ASA had long-term expression of ASA in several organs, including the brain [55]. The enzymatic activity in the brain reached 33% of the normal tissue level. Since the amount of enzyme delivered to the brain did not correlate with ASA serum levels, the authors speculate that the mechanism responsible for the gene transfer to the brain may have been migration of BM-derived cells to the brain, rather than endocytosis of serum ASA by endothelial cells.

Promising results were also obtained in a similar gene therapy strategy for MPS VII. Transplantation of MPS VII BM cells retrovirally transduced to produce GUSB in MPS VII mice led to decreased substrate accumulation, despite low-level enzymatic expression in the liver, spleen and brain [56,57]. No myeloablation was necessary in a gene therapy strategy using macrophages as target cells of gene therapy for MPS VII [58]; this is a promising result for clinical settings. When either normal syngeneic macrophages or MPS VII macrophages retrovirally transduced with human GUSB were transplanted into the mice, GUSB was detectable histochemically *in vivo* at 38 days post-transplantation, and significant pathological improvement of lysosomal storage in the liver and spleen resulted. The short life-span of terminally differentiated macrophages, and their inability to reach the brain, place limits on this strategy.

Intracranial injection of recombinant retroviral vectors

An alternative gene therapy strategy, represented by the direct delivery of the functional gene into the CNS, may be achieved by intracranial injection of recombinant viral vectors, or implantation of genetically modified cells, into the CNS. Striking results have been obtained with AAV vectors and lentiviral vectors. A stereotactically injected AAV vector containing the human GUSB cDNA achieved a broad and sustained lysosomal enzyme delivery into the striatum of adult mice severely affected by MPS VII [59]. Similar results were obtained when an HIV-derived lentiviral vector was used to reverse the pathology of the same MPS VII mouse model [60]. In mice receiving multiple injections of the GUSB-encoding lentiviral vector, complete correction or significant reduction of the pathology was observed in all histochemically stained serial sections of the brain, suggesting disease regression in the entire brain. An *in vivo* lentiviral vector gene therapy approach was also used to correct the neurological lesions of a mouse model of MLD [61•]. In addition, ERT has proven feasible to treat Sanfilipo disease murine models and the capacity of AAV for transducing MPS IIIB human fibroblasts and MPS III B mouse brain cell cultures, has been proven [22]. For this disease, direct delivery of the vector was made by stereotactical injection showing high enzymatic activities in the injected sites together with clearance of the lysosomal storage in brain cell cultures. In addition, high expression of the transgene has been reported. Lysosomal clearance from the surroundings (approximately 1.5 cm) of the injection sites support the hypothesis of bystander effect over the surrounding neural tissue which suggests that *in vivo* correction does not require the transduction of all deficient cells [23].

Implantation of genetically modified cells into the CNS

Gene transfer into the brain can also be achieved through neurotransplantation of genetically modified cells, and transduced fibroblasts and myoblasts have been implanted in the brain, although with only limited results. Taylor *et al* observed a bystander effect exerted by vector-corrected fibroblasts in the lysosomal storage in the adult MPS VII mouse brain [62]. The oligodendrocyte would be the optimal target for gene therapy of MLD. Oligodendrocytes have been transplanted into mouse models of demyelinating disease and shown to be able to survive, migrate and myelinate in the brain [63,64]. Mouse and rat neural progenitor cells have been identified which have the potential to differentiate into glial or neuronal cell types [65,66••]. These cells also retain functional properties of neuronal and glial cells *in vivo*, and do not lose their differentiation capacity when transfected with lentiviral vectors [67]. When the CG-4 cell line, a rat oligodendrocyte progenitor cell line expressing LacZ marker protein, was transplanted into the spinal cords of myelin-deficient rats, expression of LacZ was obtained in cells that had migrated along the spinal cord and showed the ability to myelinate neighboring neurons [68].

Finally, other cell types, such as amniotic epithelial cells and BM stromal cells (otherwise known as mesenchymal stem cells or MSC), have been explored as gene delivery vehicles to the CNS. An amniotic epithelial cell line engineered to produce GUSB was used as donor cell source in cell-mediated gene therapy of MPS VII, with observed improvement of the lysosomal storage in areas distant from the injection site [69]. MSCs are particularly attractive gene delivery vehicles because of their extensive differentiation potential, migration and engraftment capabilities [70-73], ease of isolation and expansion in culture [74], and because they are efficiently transduced (up to 80%) with retroviral vectors [75].

Transduction of neural progenitors is a novel therapeutic approach [76,77••,78]. Neural progenitor cells can be transduced using retroviral [76] or lentiviral (feline immunodeficiency virus, FIV) [78] vectors without differentiating into adult neural or glial cells. The migratory capacity of neural progenitor cells makes them an interesting target for transduction, which have a tropism towards tumor cells and are capable of migrating *in vitro* and *in vivo*, even when administered into peripheral vessels [78]. The widespread distribution of neural progenitor cells in the *shiverer* mouse model makes this strategy an interesting approach to treat the lysosomal storage disorders [67], particularly if the migratory capacity of neural progenitor cells is not lost, as with FIV-transfected cells [79].

These data point to the intriguing possibility of establishing a human cell line which is both transplantable to the CNS and which maintains the functional properties of oligodendrocytes. Such a cell line would offer the potential of treating MLD by gene transfer to, and transplantation of, the pathologically important cell type. In recent studies, both non-autologous neural progenitor cells and fibroblasts genetically engineered to produce GUSB were enclosed in immuno-isolating microcapsules and transplanted into the CNS of MPS VII mice, with consequent improvement of neuropathological lesions [80,81]. This strategy may overcome the immunological rejection of non-autologous cell grafts.

Neonatal transplantation

Daly *et al* showed that the need for invasive injections of viral vectors or transduced cells in the brain may be obviated if gene therapy is performed in the neonatal period [82]. An intravenous injection of GUSB-encoding AAV vector in neonatal MPS VII mice resulted in therapeutic levels of GUSB expression by 1 week of age in liver, heart, lung, spleen, kidney, brain and retina. GUSB expression persisted in most organs for the 16-week duration of the study at levels sufficient to either reduce, or completely prevent lysosomal storage. Of particular significance, neurons, microglia and meninges of the CNS were virtually cleared of disease. The authors hypothesized that the AAV vector gained entry into the brain either because of an incompletely formed BBB in neonatal mice, or because of the higher per kilogram dose of virus administered, compared with doses previously delivered to adult mice [82].

Systemic clinical disease has been prevented following AAV-mediated neonatal gene transfer in the MPS VII murine model. Treated mice showed an increased GUSB expression when compared to non-treated mice. Such expression could prevent the development of the characteristic growth abnormalities, encourage normal development in growth and phenotype, preserve retinal function and achieve partial avoidance of the development of auditory deficits. The treatment of the disease in critical stages of growth and development (first month for this mouse strain) has proven useful for preventing the development of complications, such as bone deformities, CNS, retinal or hearing abnormalities. These consequences are not reduced or cured if the disease is treated in the advanced stages [83].

Antenatal gene transfer

In many neurodegenerative storage disorders with clinical onset during infancy, there is evidence to suggest that the abnormal CNS storage of the substrate begins *in utero*. For instance, an increased concentration of sulfatide has been shown in the cerebellum, brain stem and spinal cord of a 24-week-old fetus with MLD [84]. An excess of sulfatide has also been noted in myelin isolated from another fetus with MLD [85]. In a series of electron microscopy studies in fetuses (gestational age 12 to 22 weeks) with Tay-Sachs disease, Adachi *et al* found membranous inclusions in the anterior horn cells, spinal ganglia, retina and pituitary gland [86]. In the neuronopathic variants of Gaucher disease (Type 2 and Type 3), it is possible to hypothesize that glucosylceramide accumulation in brain produces dysfunction in surrounding cells long before discrete pathologic changes are observed [87]. Advanced pathological alterations in fetal tissues, including the CNS, have been documented in the acute neuronopathic variant of Gaucher disease (Type 2) [88], which is characterized by the onset of severe disease in the fetus [89]. It is likely that these disorders not only cause degeneration of preformed neurons but also interfere with normal developmental events, with potentially irreversible consequences. BMT performed

postnatally is usually unsuccessful in reversing the neurological complications of these disorders [53]. The neurodevelopmental outcome in these patients will therefore be greatly improved by early treatment in the fetus. The neurodegenerative forms of disorders that have been corrected by postnatal BMT, such as Type I Gaucher disease [90] and Maroteaux-Lamy syndrome (MPS VI) [91], would also be candidates for prenatal transplantation of genetically modified cells. Intrauterine gene transfer should be facilitated by the immunologically permissive environment of the early gestational fetus. The clinical experience of intrauterine hematopoietic stem cell transplantation for LSDs has, however, been dismal. In five attempts to transplant fetuses with LSDs, there was little or no engraftment of donor cells and no clinical benefit (reviewed in [92]).

Two intrauterine gene therapy strategies have been evaluated in animals: the transfer of genetically engineered HSC and the direct injection of the vector. The gene-engineered HSC transplantation approach resulted in the long-term transfer and expression of the transgene at low efficiency in sheep and monkeys. In a canine model of human α-L-iduronidase deficiency (MPS I), Lutzko *et al* performed *in utero* adoptive transfer of iduronidase-transduced MPS I marrow cells into pre-immune fetal dogs. The iduronidase-transduced primitive hematopoietic progenitor cells engrafted in the fetal recipients, but no iduronidase enzyme nor proviral-specific transcripts was detected in blood or marrow leukocytes of any of the MPS I dogs [93]. Higher transduction efficiency will need to be achieved for this strategy to be effective for the treatment of human LSDs. The results of the experiments of direct injection of the vector were more encouraging. Inoculation of retroviral supernatant into the liver of fetal rats successfully transduced fetal liver HSCs, and in pre-immune fetal sheep the direct injection of viral vector into the peritoneal cavity produced long-term expression of the transgene in all tissues of the animals analyzed, including the brain (reviewed in [94]). These results are of particular interest for the development of intrauterine gene therapy approaches for LSDs with neurological involvement.

Conclusion

The medical care of LSD patients has been revolutionized by the development of effective therapeutic strategies. ERT is becoming a reality for many LSDs and the indications for BMT are being extended, as this technique becomes progressively safer. Although substrate deprivation therapy is emerging as a promising treatment for glycosphingolipidosis, such as neuronopathic Gaucher disease [95] and Sandhoffs disease (GM2 gangliosidosis) [96], there are problems limiting the implementation of this therapeutic approach to the clinical setting. Each one of these treatments has disadvantages that substantially limit its application. Most notably, the attempts at reversing the neuropathological manifestations of many LSDs have been disappointing. Hematopoietic, neural and mesenchymal stem cells are the most appealing cell subsets because of their self-renewal and differentiation potential. However, their biological behavior *in vitro* and *in vivo* has only been partially elucidated.

HSCs are readily expandable and transducible *in vitro* but their behavior *in vivo* is still unpredictable, due to the lack of extensive clinical experience. Direct viral injection may be the preferred gene therapy approach whenever systemic expression, or access to biological sanctuaries (such as the CNS) are warranted. Although promising results have been obtained in animal models of LSDs using AAV and lentiviral vectors, more preclinical testing is required prior to human clinical trials.

Promising results of gene therapy clinical trials have been shown for some diseases such as X-linked severe combined immune-deficiency (SCID) [97••], chronic granulomatous disease (CGD) [98,99], hemophilia B [100] and transfer of multi-drug resistance (MDR) [101]. However, in the case of X-linked SCID, the corrected cells had a selective advantage over the diseased counterpart, a fact that has not been seen in most LSDs.

Further clinical trials might benefit by relatively mild myeloablation of the recipients. This approach has been taken by Aiuti *et al* in two patients with adenosine deaminase (ADA)-deficient SCID [102••]. This clinical trial showed correction of the biochemical defect, engraftment of CD34+ ADA-transduced HSC and clinical improvement. Full myeloablation of patients with LSDs prior to transplantation is an unacceptable method to enhance the engraftment of transduced cells; however, the study mentioned suggests that mild myeloablation can be used for the treatment of disorders in which the transduced cells do not benefit from a selective advantage over their diseased counterparts.

Since the spectrum of the diseases is broad, it is important to draw some distinctions. In diseases such as Type I Gaucher disease, where a clinical trial showed sustained correction of the peripheral blood cells with apparent clinical benefit in one patient, a definitive gene therapy cure can be foreseen in the near future. Only incremental improvements of the gene therapy strategy employed in the study may be needed to achieve this goal. This may also be the case for Fabry disease, for which an informative animal model is available [17]. In the case of LSDs with neurological involvement, a gene therapy cure in the next few years seems an unrealistic expectation. The two main technical problems to overcome are the need to gain access to the CNS and to intervene before neurological complications have ensued. Antenatal *(in utero)* gene transfer and transplantation are interesting strategies to prevent the neuropathology in LSDs but technical problems such as cell dosing, timing of the injection and ethical questions should be solved before this will be considered a feasible gene therapy approach.

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•• of outstanding interest
• of special interest

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Inhibition of substrate synthesis as a strategy for glycolipid lysosomal storage disease therapy

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Summary: The glycosphingolipid (GSL) lysosomal storage diseases are caused by mutations in the genes encoding the glycohydrolases that catabolize GSLs within lysosomes. In these diseases the substrate for the defective enzyme accumulates in the lysosome and the stored GSL leads to cellular dysfunction and disease. The diseases frequently have a progressive neurodegenerative course. The therapeutic options for treating these diseases are relatively limited, and for the majority there are no effective therapies. The problem is further compounded by difficulties in delivering therapeutic agents to the brain. Most research effort to date has focused on strategies for augmenting enzyme levels to compensate for the underlying defect. These include bone marrow transplantation (BMT), enzyme replacement and gene therapy. An alternative strategy that we have been exploring is substrate deprivation. This approach aims to balance the rate of GSL synthesis with the impaired rate of GSL breakdown. The imino sugar *N*-butyldeoxynojirimycin (NB-DNJ) inhibits the first step in GSL biosynthesis and has been used to evaluate this approach. Studies in an asymptomatic mouse model of Tay–Sachs disease have shown that substrate deprivation prevents GSL storage in the CNS. In a severe neurodegenerative mouse model of Sandhoff disease, substrate deprivation delayed the onset of symptoms and disease progression and significantly increased life expectancy.

Combining NB-DNJ and BMT was found to be synergistic in the Sandhoff mouse model. A clinical trial in type I Gaucher disease has been undertaken and has shown beneficial effects. Efficacy was demonstrated on the basis of significant decreases in liver and spleen volumes, gradual but significant improvement in haematological parameters and disease activity markers, together with diminished GSL biosynthesis and storage as determined by independent biochemical assays. Further trials in type I Gaucher disease are in progress; studies are planned in patients with GSL storage in the CNS.

GLYCOSPHINGOLIPID STORAGE DISEASES

Glycosphingolipid (GSL) storage diseases are a family of severe, progressive disorders in which GSL species are stored in the lysosome (Figure 1) (Neufeld 1991). They have a collective incidence of 1:18 000 live births and are the most frequent cause of paediatric neurodegenerative disease (Meikle et al 1999). The diseases result from the inheritance of mutations in genes that encode acid hydrolases or their protein cofactors, which participate in the sequential removal of monosaccharide units

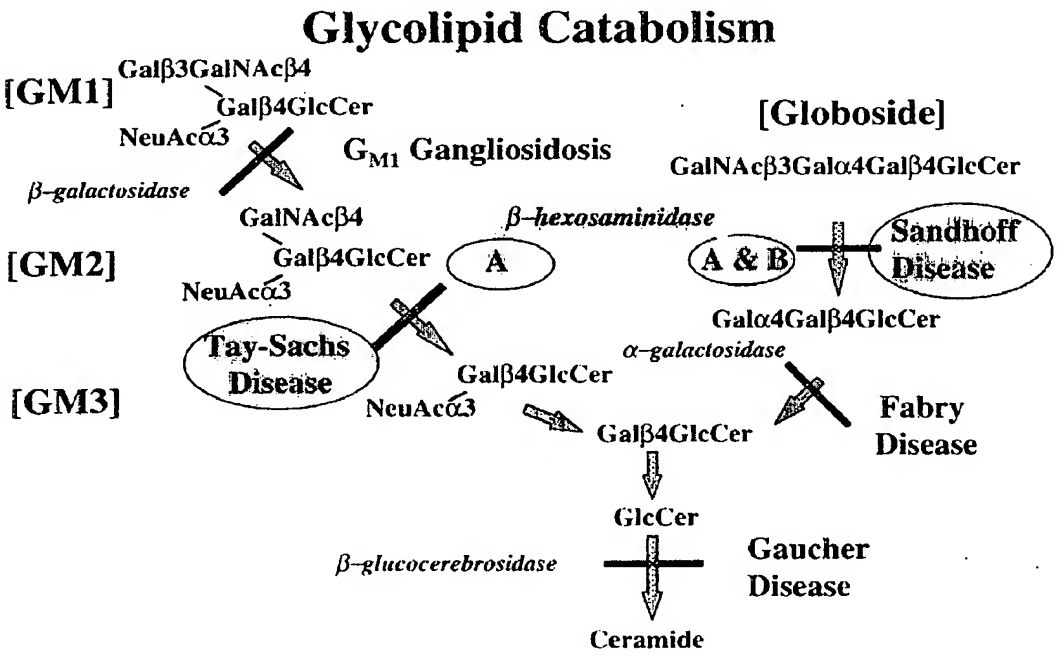


Figure 1 Summary of glycosphingolipid catabolism highlighting the enzyme deficiencies and resulting diseases. The GM2-gangliosidoses Tay–Sachs and Sandhoff diseases are emphasized because substrate deprivation therapy has been evaluated in mouse models of these two disorders

from GSLs in the lysosome (Neufeld 1991). The clinical presentation of the disease varies depending on the specific gene defect and the GSL substrate that is stored. Since there is abundant expression of multiple GSL species in the CNS, particularly gangliosides, neurological features are prominent in the glycosphingolipidoses (Walkley 1998).

THERAPEUTIC OPPORTUNITIES IN GSL STORAGE DISEASE

There are several potential approaches that can be adopted for the treatment of GSL storage diseases. Many of these strategies would be predicted to be mutually complementary or synergistic. Since these diseases are due to the inheritance of defects in the genes encoding catabolic enzymes of the lysosome, introducing a functional gene should correct the problem. In common with other human monogenic diseases, this is an approach that holds enormous promise for those afflicted with these devastating diseases (Peng, 1999; Romano et al 1999). There are currently major technical difficulties that mean that gene therapy is still at an experimental stage for these and other diseases (Marshall, 1999; SoRelle, 2000). As the majority of GSL storage disorders involve GSL storage in the CNS, delivery to the brain is a prerequisite and constitutes a formidable challenge in terms of safety and efficacy of gene delivery and expression. Small increments in lysosomal enzyme activity may be crucial in preventing or reversing the manifestations of these diseases; effective gene therapy would be predicted to be an achievable goal in many lysosomal storage diseases.

An alternative approach, which is currently established for type I Gaucher disease (and very recently considerable progress has been made towards enzyme therapy for Fabry disease (Eng et al.2000) and Pompe disease (van den Hout et al 2001), is to infuse the patient with fully functional enzyme glycoforms that are taken up by cells in target tissues (e.g. mannose-terminated glucocerebrosidase for Gaucher disease or mannose 6-phosphate containing α-glucosidase for Pompe disease). Because the passage of enzyme into the CNS is restricted by the blood–brain barrier, enzyme replacement therapy has limited efficacy in the neuronopathic variants, although the systemic manifestations of disease improve (Erikson et al 1997). An alternative approach to the development of novel treatments is to unravel the precise cellular events that take place following storage of GSLs to identify downstream intervention points. This strategy has the potential to identify targets for the action of small molecules and it is conceivable that currently used prescription drugs might have a therapeutic action in the GSL storage disease if a better understanding of their molecular pathogenesis was available. Understanding pathogenetic mechanisms has become a more realistic goal through the recent generation of mouse models of these diseases, which are amenable to detailed study and experimental manipulation (Suzuki and Mansson, 1998; Suzuki and Proia, 1998). In addition, the development of methods to investigate the gene expression profile of disease tissue using cDNA subtraction procedures or cDNA microarray 'chips' offers the possibility of establishing the pathological link between GSL storage and the protean manifestations of the glycosphingolipidoses (Moran et al 2000).

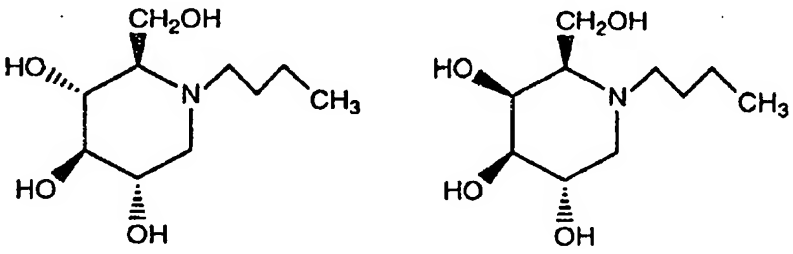
There are also cell-based therapies such as bone marrow transplantation (BMT) (which will replace haematopoietic cells with wild-type cells secreting wild-type enzyme) (Erikson et al 1990; Ringden et al 1995). The limitation here is whether the lysosomal protein in question is naturally highly secreted, for recapture by neighbouring cells. Also, the amount of brain reconstitution with bone marrow-derived microglial cells is small and this may limit the amount of functional enzyme available to cells of the CNS (Krivit et al 1995). To date, although BMT has been useful for some of these diseases, the risks associated with the procedure itself and the requirement for HLA-matched donors severely limits its clinical application.

New emerging cell-based therapies involve injection of neuronal stem cells into the brain both to serve as a source of wild-type enzyme and also to replace dead or dying cells (Svendsen et al 1999; Vescovi and Snyder 1999). This experimental approach has implications for all neurodegenerative diseases, including the GSL storage diseases (Chavany and Jendoubi 1998). Neuronal stem cell therapy remains experimental at the present time, but the availability of authentic mouse models for GSL storage diseases will facilitate the evaluation of this promising approach.

The other therapeutic option is to decrease the synthesis of the stored substrate using enzyme inhibitors. This has been termed substrate deprivation or substrate reduction therapy. This approach was first suggested by Radin and colleagues (Inokuchi and Radin 1987; Radin, 1996). The principle is very simple. If a GSL species cannot be completely degraded as a result of the inherited enzyme deficiency; the biosynthesis of fewer GSL molecules will reduce the influx of GSLs into the lysosome, allowing all molecules to be catabolized. The aim is to balance synthesis with the impaired rate of degradation. If this could be achieved, the disease process resulting from GSL storage would be arrested. If complete balance cannot be achieved, then the disease will be converted into a less severe form with a slower rate of progression. There are three major advantages to this approach; first, an oral drug could be used; second, a drug that penetrates the CNS could be utilized; and third, if an early step in the GSL biosynthetic pathway is targeted then one drug could potentially treat a family of GSL storage diseases, without the need for disease-specific intervention. Since the number of individuals with any specific GSL storage disease may be small, economic considerations related to the development of disease-specific therapy mean it is unlikely to gain pharmaceutical backing or that it will be prohibitively expensive if commercialized. However, if a family of several diseases with a relatively high collective incidence can be treated with a single drug, this then may become a viable commercial proposition. Small-molecule drugs will be cheaper than protein-based therapeutics and therefore more likely to be accessible to patients than will expensive enzyme-replacement therapy. Currently, the great majority of type I Gaucher patients treated with enzyme replacement live in affluent countries.

IMINO SUGAR INHIBITORS OF GSL BIOSYNTHESIS

In 1993 it was discovered that the *N*-alkylated imino sugar *N*-butyldeoxynojirimycin (NB-DNJ) (Figure 2) inhibited the ceramide-specific glucosyltransferase (GlcT-1,



***N*B-DNJ** ***N*B-DGJ**

Figure 2 Structures of the imino sugars NB-DNJ (glucose analogue, OGT-918) and NB-DGJ (galactose analogue)

glucosylceramide synthase, UDP-glucose-*N*-acylsphingosine D-glucosyltransferase; EC 2.4.1.80) that catalyses the first step in the GSL biosynthetic pathway (Figure 3) (Platt et al 1994a). The GlcT-1 inhibitory activity is critically dependent on a minimal *N*-alkyl chain length of three carbons (Platt and Butters, 1995; Platt et al 1994a). This compound also inhibits the *N*-glycan processing enzymes α-glucosidases I and II, which are glucohydrolases that reside in the endoplasmic reticulum (Platt et al 1992). This compound had been developed as an antiviral compound by Monsanto in the 1980s and so had been through extensive preclinical and clinical testing (Fischl et al 1994). It is orally available and very stable over a range of ambient temperatures and storage conditions (Platt and Butters, 1998). When evaluated as an anti-HIV agent in man the compound failed to show sufficient efficacy owing to the difficulty in efficiently reaching the endoplasmic reticulum (ER) α-glucosidase enzyme that was the biochemical target (Fischl et al 1994). Oral dosing to obtain a sufficiently high serum concentration of the drug was the limiting factor. If dose was escalated to try to increase ER glucosidase inhibition, the oral dose required caused extensive inhibition of GI tract disaccharidases and resulted in osmotic diarrhoea. Patient compliance was poor because of GI tract distress, which was induced by high compound dosing (Fischl et al 1994).

PRECLINICAL EVALUATION OF SUBSTRATE REDUCTION THERAPY

The discovery of the novel activity of NB-DNJ against the key enzyme in the GSL biosynthetic pathway (GlcT-1) (Figure 3) coincided with the generation by Proia and colleagues and Gravel and colleagues of knockout mouse models of Tay–Sachs (TS) and Sandhoff (SH) diseases (Phaneuf et al 1996; Sango et al 1995; Taniike et al 1995; Yamanaka et al 1994). NB-DNJ was therefore evaluated in these mouse models (Sango et al 1995) to answer two critical questions: (a) would sufficient NB-DNJ cross the blood–brain barrier to slow storage in the asymptomatic TS mouse; and (b) in a symptomatic neurodegenerative mouse model (SH), would the disease process be significantly slowed by NB-DNJ therapy?

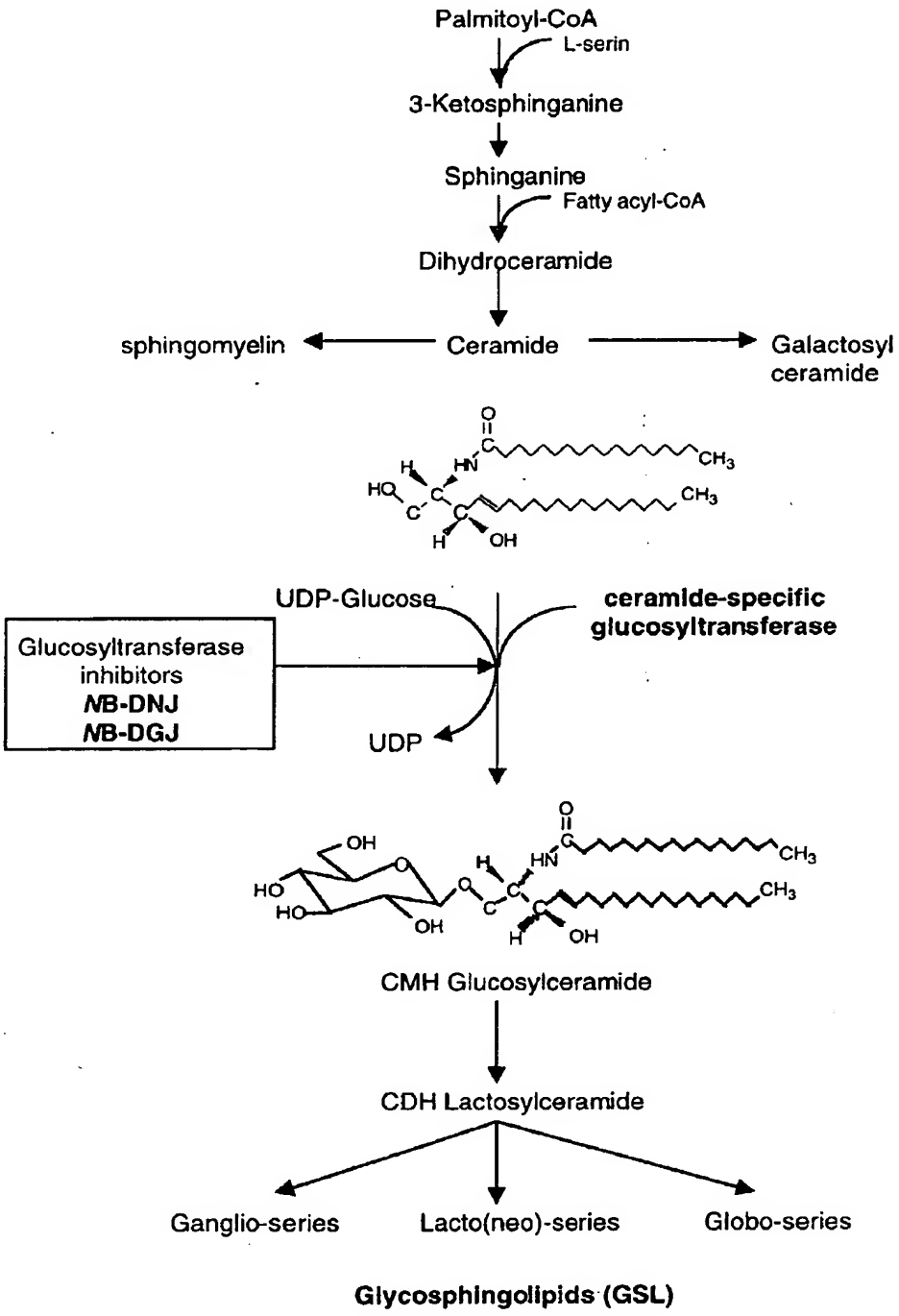


Figure 3 Summary of glycosphingolipid biosynthesis highlighting the glucosyltransferase-catalysed biosynthesis of GlcCer, which is the step in the pathway inhibited by NB-DNJ and NB-DGJ

MOUSE MODEL OF TAY–SACHS DISEASE

In the mouse model of Tay–Sachs disease (generated by the targeted disruption of the mouse *Hexa* gene), the mice store GM2 ganglioside in a progressive fashion, but the levels never exceed the threshold required to illicit neurodegeneration (Taniike et al 1995; Yamanaka et al 1994). This is because in mice (but not in humans) a lysosomal sialidase is sufficiently abundant or active to convert GM2 to GA2, which can then be catabolized by the hexosaminidase B isoenzyme, which is unaffected by the *Hexa* knockout (Sango et al 1995).

To evaluate substrate deprivation in the Tay–Sachs mouse model, mice were reared on food containing NB-DNJ (Platt et al 1997a). The pharmacokinetics of NB-DNJ are two orders of magnitude poorer in mice than in humans. This necessitates higher dosing in mice to achieve serum levels (5–50 μmol/L) in the predicted therapeutic range (partial inhibition of GlcT-1) for the GSL storage disorders (Platt et al 1997a). The mice were monitored for 12 weeks; a reduction in stored GM2-ganglioside was observed in all animals from the NB-DNJ-treated group (50% reduction in GM2- ganglioside in the brains of treated mice relative to the untreated controls). In GSL storage regions of the brain, the NB-DNJ-treated mice had fewer periodic acid–Schiff (PAS)-positive neurons (PAS detects the stored GM2) and the intensity of staining in each neuron was reduced relative to that in those of untreated age-matched controls (Platt et al 1997a). At the EM level, in storage neurons from untreated Tay–Sachs mouse brains, prominent regions of the cytoplasm contained large numbers of membranous cytoplasmic bodies (MCBs) containing stored GM2. In contrast, in the NB-DNJ-treated mice, storage neurons were scarce. When storage cells could be identified they contained MCBs that had greatly reduced electron density. NB-DNJ was therefore able to cross the blood–brain barrier to an extent that prevented storage (Platt et al 1997a).

The finding that GSL depletion can be achieved in the CNS is significant because all the GlcCer-based GSL storage diseases could potentially be treated with NB-DNJ (Platt and Butters, 1998). NB-DNJ does not, however, inhibit the galactosyltransferase that initiates the biosynthesis of galactosylceramide (GalCer)-based GSLs. This is significant because the synthesis of GalCer and sulphatide, which are important components of myelin, will not be affected by NB-DNJ treatment and therefore myelination and myelin stability should not be impaired (Platt et al 1997a). As a consequence, NB-DNJ would not be predicted to be effective in the treatment of Krabbe disease and metachromatic leukodystrophy, as both of these diseases involve the storage of GalCer-based GSLs (GalCer and sulphatide, respectively) (Neufeld 1991).

EFFECTS OF NB-DNJ IN MOUSE MODEL OF SANDHOFF DISEASE

The mouse model of Sandhoff disease was generated through the targeted disruption of the *Hexb* gene and lacks hexosaminidase A and B isoenzymes, resulting in the storage of GM2- and GA2-gangliosides in the CNS and periphery (Sango et al 1995). The Sandhoff disease mouse has very low levels of residual enzyme activity, con-

ferred by the minor hexosaminidase S (αα) isoenzyme. The mice undergo rapid, progressive neurodegeneration and die at 4–5 months of age (Sango et al 1995). When Sandhoff mice were treated with NB-DNJ, their life expectancy was increased by 40% and GSL storage was reduced in peripheral tissues and in the CNS (Jeyakumar et al 1999). Following the onset of symptoms, the rate of decline was significantly different in untreated and NB-DNJ-treated mice, as was the age at which deterioration could first be detected (approximately 100 days for untreated mice and approximately 135 days for NB-DNJ–treated mice). However, the terminal stage of the disease (when the mice are moribund) was prolonged in NB-DNJ-treated mice. When GSL storage levels were measured in the untreated and NB-DNJ-treated Sandhoff mice at their end points (at 125 days and 170 days, respectively), the levels of GM2 and GA2 were comparable, indicating that death correlated with the same levels of GSL storage in the brains of the two groups of mice. Histological examination of the mice at 120 days showed reduced storage in the brain of NB-DNJ-treated mice. At the ultrastructural level, the neurons showed greatly reduced storage burdens. This reduction in GSL storage was even more pronounced in the liver. The liver, like other peripheral organs, is exposed to higher concentrations of NB-DNJ, whereas only about 5–10% of the concentration in the serum is detected in the cerebrospinal fluid (Platt et al 1997a).

COMBINATION THERAPY IN THE SANDHOFF MOUSE

Both NB-DNJ therapy (Jeyakumar et al 1999) and BMT (Norflus et al 1998) increase life expectancy in the Sandhoff mouse. The main factor that limits the efficacy of NB-DNJ treatment is the lack of significant residual enzyme levels in this mouse model. BMT is limited by the fact that few donor origin cells repopulate the brain. Combining these two approaches would be predicted to be complementary and potentially synergistic.

We have therefore evaluated the efficacy of combining these two therapies. Sandhoff disease mice treated with BMT and NB-DNJ survived significantly longer than those treated with BMT or NB-DNJ alone. When the mice were subdivided into two groups on the basis of their donor bone marrow-derived CNS enzyme levels, the high-enzyme group exhibited a greater degree of synergy (25%) than did the group as a whole (13%). Combination therapy may therefore be the strategy of choice for treating the infantile-onset disease variants (Jeyakumar et al 2001).

CLINICAL EVALUATION OF SUBSTRATE DEPRIVATION THERAPY IN TYPE I GAUCHER DISEASE

In 1998–1999 patients with nonneuronopathic Gaucher disease were recruited at four centres (Cambridge, Amsterdam, Prague and Jerusalem) into a one-year open-label clinical trial of NB-DNJ (Cox et al 2000). The trial was coordinated by Oxford GlycoSciences and NB-DNJ was referred to as OGT-918.

Twenty-eight adult patients (14 females and 14 males) were enrolled, seven of whom had had previous splenectomies. All patients were unable or unwilling to take

enzyme replacement therapy. Liver and spleen volumes were measured by MRI or computed tomography and haematological parameters were monitored. In addition, several biochemical markers were measured, including chitotriosidase (Aerts and Hollak 1997), cell surface leukocyte GM1 as an indicator of whether GSL levels were depleted in response to OGT-918 treatment, and the plasma levels of GlcCer, the storage lipid.

Most patients were treated with oral doses of 100 mg OGT-918 three times per day. Three patients received 200 mg three times a day and four patients had their doses lowered to 100 mg once or twice a day. The rationale for individualized dosing was based upon individual variation in the pharmacokinetics of the compound, tolerability and organ volume response after 6 months of treatment.

Pharmacokinetics

On the basis of the *in vitro* studies (Platt et al 1994a,b), normal mouse studies (Platt et al 1997b) and animal models studies (Platt et al 1997a), it was thought that a serum level in humans of 5–10 μmol/L should be sufficient to partially inhibit GSL synthesis and impact the disease (Platt and Butters 1998). Pharmacokinetic profiling in a subgroup of patients showed that the drug reached maximum plasma concentrations by 2.5 h with a plasma half-life of 6.3 h. Steady-state concentrations of OGT-918 were achieved by day 15 of dosing and the mean peak level of OGT-918 over the 12-month study was 6.8 μmol/L with trough values of 3.9 μmol/L (Cox et al 2000).

Side-effects

The major known side-effect of OGT-918 is diarrhoea. This was noted in the previous trial with this compound when it was tested as an antiviral agent (Fischl et al 1994). The compound is a disaccharidase inhibitor and therefore prevents the breakdown of complex dietary carbohydrates at the intestinal brush border. Unabsorbed sugar molecules remain in the gastrointestinal tract, leading to the osmotic influx of water into the intestinal lumen and resulting in diarrhoea and flatulence due to enhanced bacterial fermentation.

In the Gaucher clinical study the dose given was tenfold lower than in the HIV trial. In the Gaucher study it was found that, although most patients reported GI tract symptoms as soon as they started taking OGT-918, the diarrhoea spontaneously resolved in most patients within several weeks and did not generally pose a significant problem (Cox et al 2000).

Of the 28 patients enrolled in the trial, 6 withdrew. Two were unable to tolerate the GI tract side-effects (one suffered from Parkinson disease and the other did a lot of business travel). Two patients withdrew owing to pre-existing medical conditions (hepatocellular carcinoma and pulmonary hypertension), one additional patient left to start a family and another was advised by her rabbi to withdraw after one day on study. The remaining 22 patients were monitored at 6 and 12 months for signs

of clinical improvement. Two further patients withdrew because of symptoms of peripheral neuropathy. All other patients on OGT-918 have been investigated by electromyography and to date no other cases have been identified. Eighteen patients have continued to receive OGT-918 beyond the 12-month study in an extended treatment protocol, with some patients having so far taken therapy for 2.5 years.

Biochemical efficacy

One of the critical issues concerning the use of OGT-918 in man was whether GSL depletion can be achieved. The activity of NB-DNJ to inhibit GSL biosynthesis was unknown when the HIV clinical trial was conducted, so this property of the drug had never been investigated in humans prior to the Gaucher clinical trial.

GSL depletion was assessed in three different ways. First, a GSL unrelated to the disease was monitored on the cell surface of leukocytes to give a sensitive measure of general GSL depletion. This was achieved using a flow cytometric assay measuring cell surface GM1 (Platt et al 1994a). This demonstrated a 38% reduction after 12 months of therapy (Cox et al 2000). On a small number of samples, levels of leukocyte LacCer (a lipid that contributes to the GlcCer storage burden in macrophages owing to its abundance in the cells they phagocytose) were measured by TLC and also showed a time-dependent reduction (F. Platt and T. D. Butters, unpublished data). Finally, preliminary analysis of GlcCer itself was done in the plasma of several patients and initial analysis demonstrated reduced levels following treatment (H. J. Aerts and S. van Weely, unpublished data). Taken together, these data show that, at the plasma levels achieved in the study, (a) GSL expression is reduced in accordance with the mechanisms of action of this drug; (b) LacCer in leukocytes is reduced, thereby reducing the burden of this lipid ingested by macrophages; and (c) the disease storage product (GlcCer) present in the plasma was reduced from baseline. This therefore provides the biochemical foundation for the proposed substrate deprivation mechanism central to this treatment strategy. Furthermore, when the plasma was investigated for the presence of glycosylated *N*-glycans that arise due to ER α-glucosidase inhibition (the activity of NB-DNJ responsible for its antiviral properties), only trace levels could be detected. Therefore, as predicted from previous studies (reviewed in Platt and Butters 2000), the low dose of compound used in the Gaucher trial has little impact on the other pathway inhibited by this drug because of the inaccessibility of the α-glucosidase-1 enzyme target (located in the ER lumen).

Clinical efficacy

Spleen and liver volumes showed a statistically significant reduction (15%, range 11.8–18.4, $p<0.001$ and 7%, range 3.4–10.5, $p<0.001$, respectively) after 6 months of therapy. At 12 months the decrease from baseline was 19% (range 14.3–23.7,

$p<0.001$) and 12% (range 7.8–16.4, $p<0.001$), respectively (Cox et al 2000). When chitotriosidase, a marker of the disease activity, was measured, the macrophage-derived enzyme showed a time-dependent reduction, indicating a reduction in the total pool of Gaucher cells within the patients treated with OGT-918 (Cox et al 2000). Haematological parameters of haemoglobin and platelet counts showed trends towards improvement, with a greater improvement in haemoglobin noted in patients who were anaemic at baseline. A statistically significant improvement in platelet counts was achieved following 12 months of treatment. Assessment of 18 patients in the extended-use protocol has shown (a) continued improvement in organ volume reduction, (b) further haematological improvements in platelets and haemoglobin (all values now statistically significant) and (c) continued decline in chitotriosidase (presented at the 4th EWEGD workshop, Jerusalem, September 2000 by Zimran et al). These data strongly indicate that GSL depletion improves all key clinical features of Gaucher disease.

Kinetics of clinical improvement

Substrate deprivation therapy was predicted to be slower in its effects than enzyme replacement therapy owing to the different mechanisms of action of these two agents. Indeed, this was the rationale for choosing an initial 12-month trial period. In Gaucher disease the main pathology is the result of Gaucher cells, which are macrophages engorged with GlcCer. Although the enzyme deficiency is present in every cell type, the manifestations are restricted to professional phagocytes. The reason for this is that reduced glucocerebrosidase activity in nonphagocytes is tolerated as more enzyme is expressed in the lysosome than is needed to cope with the normal turnover of endogenous GSLs in the cell. However, in the case of the macrophage, the residual enzyme cannot cope with the burden of GSLs acquired following phagocytosis of apoptotic leukocytes and senescent red blood cells. Hence, the storage occurs selectively in macrophages, leading to their activation and the resultant pathology. If substrate deprivation is to work it therefore requires that leukocytes and red cells are produced in the bone marrow in the presence of NB-DNJ to inhibit GSL biosynthesis, thus lowering their GSL content. When these cells are finally ingested by macrophages, the GSL burden entering the phagolysosome will be decreased. As the life-span of the cells in questions is in the range of several weeks to months, we expected that the response to OGT-918 would be slower than that observed with enzyme replacement. This was borne out in particular by the slower rate of haematological improvement and the slightly slower reduction in organ volumes and plasma chitotriosidase activity. It is interesting that the decrease in organ volumes occurred more rapidly than the haematological improvement. The reasons for this are not yet known. However, in the analysis of the data from the Gaucher registry, analysing data from over 700 Gaucher patients, it was found that the degree of splenomegaly did not correlate with the degree of cytopenia. These two events are therefore not directly coupled (Scott et al 2000). The rate of response to NB-DNJ therapy also suggests

that the pathological effects of storage are not the same in all sites of disease. It appears that reduction in the influx of GSLs into macrophages of spleen and Kupffer cells in liver is more rapidly affected than it is in bone marrow. As the precise sites of apoptotic cell clearance and the nature of the cells being phagocytosed in the different sites are still under active investigation, the cause of the differential responses must remain speculative. However, the turnover rates of cells in the bloodstream differ greatly and they may well have preferential sites of individual clearance.

One of the clear predictions of the model proposed by Conzelmann and Sandhoff (1983) was that small differences in residual enzyme activity would make a big impact on storage. Substrate deprivation lowers substrate influx into the lysosome, allowing the residual enzyme to degrade newly incoming glycolipid and ultimately clear the stored material. Patients who have taken many years to develop symptoms (typical type I Gaucher patients) are storing GSL very slowly at the onset of disease, implying that they are only just below the critical threshold of residual enzyme required to escape pathology. A very small shift in synthetic rates of GSLs in these individuals would be predicted to allow residual enzyme to catabolize the storage material in the same way that enzyme replacement does. In this case, however, the enzyme is already in the lysosome and can therefore efficiently start degrading stored glycolipid. The probable difference in the kinetics of visceral disease improvement (as judged by changes in organ volume) and improved bone marrow function (as judged in part by haematological changes) may reflect the different cellular sources of stored GSL. For instance, T cells are known to die in the liver either by homing to the liver following commitment to die by apoptosis or by traficking to liver, where the death pathway is initiated (Crispe and Mehal 1996). There is a degree of controversy about where cells of different lineages die and how they are recognized and phagocytosed. Bone marrow phagocytes probably deal with clearance of cells that die *in situ*, whereas the liver serves as a major clearance site for peripheral T cells (Crispe and Mehal 19996). The spleen is likely to be the site of clearance of senescent platelets, other leukocytes and red cells. As the rates of phagocytosis (i.e. the number of cells phagocytosed per macrophage per day, and therefore the phagocytic GSL burden) and the half-lives of the various circulating cell types are different, it is not surprising that there is a hierarchy of organ involvement and response to therapy. Clearly, enzyme replacement will impact all of these simultaneously as the only prerequisite is that macrophages in different sites are all accessible to enzyme and that sufficient is delivered to the lysosome. In the case of substrate deprivation, many unknown factors could influence the rate of clinical improvement in a given organ including (a) rate of phagocytosis in the organ, (b) lineage of phagocytosed cells, (c) half-life of phagocytosed cells, (d) glycolipid content of phagocytosed cell(s) and (e) heterogeneity of the lipid components and their individual contributions to the pathological cascade that is responsible for the complex manifestations of Gaucher disease. A viable mouse model of Gaucher disease would greatly facilitate the investigation of these questions and assist our understanding of the pathogenesis of Gaucher disease.

PROSPECTS FOR COMBINATION THERAPY IN GAUCHER DISEASE

In principle, it would be predicted that, in just the same way that BMT and substrate deprivation are synergistic in their action in the Sandhoff mouse model (Jeyakumar et al 2001), combining intravenous enzyme replacement and substrate deprivation in Gaucher patients would be a rational treatment option. Multiple permutations could be envisaged, including monotherapy, sequential therapy (i.e. enzyme followed by NB-DNJ maintenance) or co-administration (i.e. continuous NB-DNJ with periodic enzyme administration). One issue that would restrict direct co-administration would be inhibition of glucocerebrosidase by NB-DNJ, as this compound is a known inhibitor of this enzyme. The IC_{50} value for β-glucocerebrosidase inhibition is 520 μmol/L which is 25 times higher than that required to inhibit the ceramide-specific glucosyltransferase (IC_{50} 20 μmol/L) (Platt et al 1994b). Therefore, in the presence of a serum concentration of NB-DNJ of 5–50 μmol/L, NB-DNJ will inhibit GSL biosynthesis but not cause inhibition of glucocerebrosidase (Platt et al 1994b). This has now been demonstrated *in vivo* in healthy mice. When mice were treated with NB-DNJ 4800 mg/kg per day (50 μmol/L serum level) and co-administered glucocerebrosidase (5–10 U/kg Ceredase), no inhibition of enzyme was detected (even at inhibitor concentrations above those being achieved in the clinical studies) (Priestman et al 2000). If anything, apparent potentiation of circulating enzyme half-life was observed. This increase in circulating half-life could be due either to reduced enzyme uptake or to stabilization of the enzyme. Glucocerebrosidase is taken up via macrophage mannose receptors, which do not recognize glucose. It is therefore very unlikely that a glucose analogue such as NB-DNJ would directly bind to this receptor. However, the enzyme is unstable at neutral pH and is rapidly inactivated in the plasma, and it therefore seems more likely that the inhibitor stabilized the active site of the enzyme in an analogous fashion to the stabilization of α-galactosidase by DGJ (Fan et al 1999). If enzyme half-life is extended in man, it may promote uptake over time into macrophages, as the vast majority of enzyme administered is not taken up by macrophages. Irrespective of the mechanism and whether or not enzyme potentiation is observed in man, co-administration is a viable option, as no enzyme inhibition occurs, and may increase considerably the therapeutic options for the management of this particular disease.

FUTURE COMPOUNDS FOR SUBSTRATE DEPRIVATION THERAPY

The compound NB-DGJ is a galactose analogue that is also a potent inhibitor of the ceramide glucosyltransferase (but not the galactosyltransferase important in Gal-Cer synthesis for myelin function). We have compared this analogue to NB-DNJ both in *in vitro* enzyme assays and also at high doses in mice to unmask potential side-effects. NB-DGJ is a more selective enzyme inhibitor with fewer complicating inhibitory properties and will be subjected to preclinical toxicological testing in the near future (Andersson et al 2000). One very important observation was made in this comparative study. It was found that none of the effects of NB-DNJ

induced at high doses (10 times greater serum level than the clinical study), such as weight loss and lymphoid organ shrinkage (Platt et al 1997b), are attributable to GSL depletion. They must be due to other properties of NB-DNJ, since NB-DGJ lacks these effects but is an equivalent inhibitor of GSL biosynthesis *in vivo* (Andersson et al 2000).

It has also been shown that deoxynojirimycin with a hydrophobic adamantane group linked via a pentyl spacer is an extremely potent inhibitor of GlcCer synthesis in cultured cells (IC_{50} of approximately 50 nmol/L) (Overkleeft et al 1998). It remains unclear whether this type of very hydrophobic compound is intrinsically suited for medical applications or is only of value as a tool in the fundamental research of GSL synthesis and transport.

FUTURE FOR CNS THERAPY

The clinical study in type I Gaucher disease has provided evidence for improvement in many signs and laboratory features of the disease. An increase in therapeutic options for type I Gaucher disease could provide alternative regimes for treating patients. The fact that two therapeutic approaches (enzyme replacement and substrate deprivation) attack the disease from different mechanistic sides of the synthesis : catabolism equation should permit a whole range of treatment and management protocols to be devised and evaluated clinically.

However, the principal additional contribution that the substrate deprivation approach could make would be in the currently refractory and severe variants of Gaucher disease (types II and III), which affect the brain, and in the gangliosidosis patients who have progressive neurodegenerative disease. The preclinical studies in mouse models of Tay–Sachs and Sandhoff disease (Jeyakumar et al 1999; Platt et al 1997a) offer the prospect that these drugs may be of benefit to patients with these conditions, at least those with the juvenile- and adult-onset variants of these disorders. The intractable infantile-onset variants will undoubtedly need an additional enzyme augmenting modality if the disease is to be treated (Jeyakumar et al 2001).

With the advent of more effective means for delivering enzymes to the CNS (BMT, gene therapy and neuronal stem cell therapy), there is a real prospect that over the next decade there may be a number of strategies available for improving the lives of the patients suffering from these devastating neurological diseases.

ACKNOWLEDGEMENTS

F.M.P. is a Lister Institute Research Fellow. R.H.L. is a Wellcome Trust Clinician Scientist Fellow.

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Neurochemical Research, Vol. 24, No. 4, 1999, pp. 601–615

Gene Transfer Approaches to the Lysosomal Storage Disorders*

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(Accepted August 28, 1998)

The work summarized in this paper used animal and cell culture models systems to develop gene therapy approaches for the lysosomal storage disorders. The results have provided the scientific basis for a clinical trial of gene transfer to hematopoietic stem cells (HSC) in Gaucher disease which is now in progress. The clinical experiment is providing evidence of HSC transduction, competitive engraftment of genetically corrected HSC, expression of the GC transgene, and the suggestion of a clinical response. In this paper we will review the progress made in Gaucher disease and include how gene transfer might be studied in other lysosomal storage disorders.

KEY WORDS: Lysosomal; genes; transfer; storage; disease.

INTRODUCTION

Transfer of the Glucocerebrosidase Gene to Mouse Hematopoietic Cells. We have successfully transduced murine whole bone marrow with high efficiency using a retroviral vector (MFG-GC). Analyses of the tissues of mice reconstituted with transduced bone marrow revealed that the efficiency of gene transfer was essentially 100%. At 4-8 months post transplant, bone marrow, spleen, thymus, and lymph node were completely repopulated by donor cells and each cell contained 1-2 copies of the transgene. Other organs including liver and lung also were completely reconstituted with their usual complement (−10%) of bone marrow derived cells. These results conclusively demonstrated that murine bone marrow contains rapidly dividing stem cells which can be targeted efficiently by a retroviral vector carrying the GC gene. In addition, the vector is capable of expressing the GC gene in a robust fashion. Enzymatic analyses for GC activity in the tissues of transplanted animals showed that the activity was 4-5 times the control levels. The results demonstrate that the transgene produces activity in an amount that is likely to be therapeutic in patients with Gaucher disease.

In addition, we have performed secondary bone marrow transplants with the marrow obtained from the primary transplants described above. Animals receiving these transplants have been analyzed for the presence and expression of the human transgene. Spleen colonies at 12 days post transplant were all positive for the human transgene. Extracts of the colonies were assayed for enzymatic activity. All showed activities that were 4-5 times higher than the activities of control animals transplanted with bone marrow that had not been transduced by the human GC gene. A group of secondary transplant animals were kept for more than 1 year post transplant. The leukocytes in their blood were analyzed for enzymatic activity. The cells showed that the transferred gene expressed GC at levels 4-5 times control levels. These results show conclusively that bone marrow stem cells had been transduced. Their progeny–the circulating leukocytes–carried and expressed the human gene essentially for the life of the mice.

Our studies demonstrate several important points which were not certain prior to these experiments. First,

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* Special issue dedicated to Drs. Ann and Hugo Moser.

0364-3190/99/0400-0601$16.00/0

the efficiency of stem cells transduction by a retroviral vector was not known to be so high. A few studies had suggested this possibility, but none had conclusively demonstrated this point in vivo. The demonstration of the transgene in every spleen colony in secondary transplants had not been shown previously in any model including reports of transfer of the GC gene. Second, expression of the GC transgene has not been accomplished to the same extent using any other retroviral vector in vivo studies. We obtained enzyme activities that were 4-5 times control levels in every tissue that we examined. No other vector has achieved this degree of expression of the GC gene. Third, it had been thought that the retroviral promoter/enhancer was shut down in vivo after a short time in hematopoietic cells. Our studies showed no abatement of expression in animals for more that 1 year. Our results clearly show that the LTR continues to function in vivo for at least 12 months.

It is also an important result that the transduced HSC engraft. Studies by other investigators suggest that transduced HSC acquire an engraftment defect. This problem was not encountered. Moreover, the combination of high transduction efficiency and engraftment of transduced HSC results in complete reconstitution of ablated animals with genetically modified bone marrow.

Our studies have important implications for the potential of gene therapy for Gaucher disease. In the murine model, gene transfer to stem cells is efficient, transduced stem cells engraft, and the GC transgene is expressed at high levels for life. On the basis of these and other studies in human hematopoietic cells, we were approved to conduct a study of gene therapy in patients with Gaucher disease.

We have continued to examine the ability of the MFG retroviral vector to transduce and express in hematopoietic stem cells (HSC) and their progeny employing the mouse model of bone marrow transplantation. Previously we have shown that the MFG-GC construct carrying the human glucocerebrosidase cDNA (GC) was remarkably efficient in transducing an oligoclonal population of bone marrow reconstituting cells. We demonstrated that the HSC responsible for reconstituting the bone marrow were transduced with nearly 100% efficiency. In addition, expression of the human GC gene was at least four fold higher than background activity and persisted for the life of the transplanted animals. We conducted experiments to study if the features of high efficiency transduction and robust expression were reproducible in other MFG constructs or were particular to the MFG-GC vector alone. In these studies, we constructed an MFG retroviral vector with an entirely different cDNA ligated to the NcoI site at the ATG of the deleted env gene of the MMLV derived vector. The cDNA was a clone for arylsulfatase A (ASA). In the retroviral plasmid, MFG-ASA, was transfected into the ecotropic packaging line, psi-cre. The highest producing clones were selected and used to infect the amphotrophic producer, psi-crip. The vector producing cell line, MFG-ASA/psi-crip, was used to infect murine bone marrow by co-cultivation in a system using recombinant cytokines: IL-3, IL-6, and SCF. These transduced bone marrows were transplanted to lethally irradiated recipient mice. Some of the recipients were evaluated for carriage and expression of the human ASA transgene in spleen colonies at twelve days post transplant. Other transplant recipients were kept for four months or more and then sacrificed to evaluate carriage and expression of the transgene in bone marrow, spleen, thymus, lymph node, liver, and brain. The results of these measurements showed that the ASA gene was efficiently transferred to the oligoclonal HSC responsible for short term (CFU-S) and long term reconstitution of bone marrow. Further, the expression of ASA was routinely two fold greater than the control levels of the enzyme in murine cells and tissues. In addition, cells carrying the ASA transgene could be identified in the brain of long term reconstituted animals. These results closely parallel those that we obtained using MFG-GC. The experiments demonstrate that the MFG vector performs in a fashion that is not specific to the transgene. The construction is predictable in terms of its ability to express the transgene. Construction and use of MFG-like vectors by other groups have not shown similar advantages in terms of expression. We conclude that the steps used in other laboratories in cloning into MFG are subtly different or that the MFG-like vector itself is different such that some important feature of the MFG vector is altered or lost. These differences result in less efficient expression of the passenger transgene. We will utilize the MFG ASA vector to study the ability of genetically corrected ASA bone marrow to replenish the deficiency of ASA in the brain of transgenic mutant mice provided to us by our collaborator.

Gene Transfer in the Gaucher Mouse Model. In preparation for studies of the Gaucher mouse, we have examined the transduction of whole mouse bone marrow by virus-containing supernatants. In our studies in normal mice, reported above, the marrow was transduced by co-cultivation. In order to simulate the protocol to be used in human studies, we have examined transduction efficiency using a supernatant infection protocol employing centrifugation. Similar transduction efficiencies were obtained.

We have examined centrifugation as a method to enhance the transduction of $CD34^+$ cells. Using TF-1

cells, stable expression of the glucocerebrosidase (GC) gene can be demonstrated for months in culture at high levels. With cord blood $CD34^+$ cells as the targets, transduction efficiency is on average at 50%. This result is considerably better than results published by other investigators.

Transduction of hematopoietic cells for a clinical autologous transplant of genetically corrected cells is a logistical problem. Even the $CD34^+$ enriched fraction of mobilized peripheral blood stem cells (PB SC) requires time and materials that prohibit the conduct of a clinical experiment using traditional methods. Approximately 2×10^8 $CD34^+$ cells are required to meet the specifications of the FDA-approved protocol. To overcome this impediment, study of transduction of $CD34^+$ cells by centrifugation in blood transport bags was conducted on $CD34^+$ cells obtained in an IRB-approved experiment on three patients with Gaucher disease. A transduction efficiency of up to 50% was demonstrated.

In order to determine if the transduced cells expressing GC activity are capable of engraftment, we have initiated two additional assays that are helpful in estimating engraftment. If positive, these assays would support the engraftment data obtained in the patients summarized below. The two assays are the SCID-hu bone assay and the high proliferative potential-colony forming unit (HPP-CFU) assay.

In the SCID-hu bone assay, human fetal bone is engrafted in a NOD-SCID mouse. This eliminates the need for high daily doses of human cytokines-more than $10,000 for a single study of five mice. It is also efficient in that only 10,000 human cells are needed to inject the bone to study engraftment and proliferation of stem cells. We are using this model to study the percentage of genetically corrected $CD34^+$ cells that engraft in the mouse. Samples of the patient $CD34^+$ cells that were transduced are used in the model. In this parallel assay, the results obtained in the patient can be compared to the engraftment in this surrogate model. Alteration of the clinical protocol to improve transduction and engraftment (discussed in the next section) can be evaluated in the model. This approach should provide substantially more data on which to base conclusions on the success of the strategies tested.

In the HPP-CFU assay, $CD34^+$ cells are placed in a culture system that provides a characteristic appearance to the cells grown in it. These characteristic cells have been shown to have a high proliferative potential in mouse models of bone marrow reconstitution. The assay is, in this sense, an estimate of stem cells. A correlation between the assay and human marrow engraftment has not been made. Our clinical trial will provide that opportunity. We will use the assay to measure the number of HPP-CFU in the transduced $CD34^+$ cells population for the patient sample. Analysis of the HPP-CFU is done by PCR for the GC transgene in the vector. We have also demonstrated a correlation between the TE of HPP-CFU and the TE of CFU-GM. This supports the use of the CFU-GM assay as a surrogate marker for the determination of HPP-CFU transductions.

A high transduction efficiency of HPP-CFU would indicate the high probability of stem cell transduction using the same HPP-CFU assay in mice and estimating reconstitution by competitive engraftment. We plan to evaluate strategies to improve engraftment of transduced stem cells. In essence, the plan is 'to stimulate' stem cells into division, transduce them, then culture them in a combination of cytokines that permits their engraftment. The results of these studies should permit the prediction of a set of conditions that would be useful for the transduction and engraftment of human stem cells. This strategy may increase the total number of transduced stem cells in culture. We will evaluate this approach using the CFU-GM, HPP-CFU, and SCID-hu bone assays.

Clinical Trial of Gene Therapy for Gaucher Disease. Transplants of genetically-corrected, autologous $CD34^+$ cells have been performed. All of the subjects were able to mobilize $CD34^+$ cells using G-CSF. Sufficient $CD34^+$ cells to achieve a dose of 2×10^6 cells/kg were able to be harvested in two leukopheresis procedures performed on consecutive days. Cryopreservation of the $CD34^+$ cell harvest was not required. Transduction efficiency averaged 20% and has improved consistently with adjustments to the protocol. The activity of glucocerebrosidase in the transduced cells averaged 150 units/mg protein. This is a ten fold increase above the deficient levels and returns the activity of GC to normal levels.

Total peripheral blood leukocytes and $CD34^+$ cells prepared from the blood of study subjects are positive by PCR for the GC transgene. The GC activity of total PBL and isolated $CD34^+$ cells has increased gradually following the transplantation of transduced $CD34^+$ cells. In the longest studied subject, the GC activity of total PBL has risen to a level typical of a heterozygote.

These results are the most impressive produced to date in gene transfer approaches to hematopoietic cells in terms of competitive engraftment of transduced progenitors, transduction efficiency, and expression of the transgene to levels that may be therapeutic. In one study subject, the dose of glucocerebrosidase has been reduced to zero in a stepwise fashion over 12 months without evidence of a clinical reversal.

Study of Gene Transfer in Metachromatic Leukodystrophy. The overall goal of the proposed studies is to study the feasibility and potential efficiency of gene transfer as an approach to the treatment of metachromatic leukodystrophy (MLD).

Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder of sulfatide metabolism adversely affecting the maintenance of myelin. Individuals with MLD are deficient in the activity of arylsulfatase A (ASA), a lysosomal enzyme which hydrolyzes galactosyl sulfatide (cerebroside sulfate) (1). This single gene defect gives rise to lysosomal storage and accumulation of sulfatide in the white matter of the central nervous system (CNS) and peripheral nerves, and to a lesser extent the visceral organs. Sulfatide-induced changes are responsible for the loss of myelination in the CNS, and a range of ensuing neurologic deficits which result in neurologic deterioration and death (2). Molecular cloning of the human cDNA and determination of the gene structure (4,5) have led to the identification and characterization of many mutant alleles and correlation's of genotype with MLD phenotype (1,6). MLD-related mutations fall into two broad groups that incompletely correlate with the clinical phenotype. The first group (I) produces no active enzyme, no immunoreactive protein, and expresses no ASA activity when introduced into cultured animal cell lines. The second group (A) generates small amounts of cross-reactive material and low levels of functional enzyme in cultured cells. Individuals homozygous for an I group mutation express infantile MLD. Most individuals with one I-type and one A-type mutation develop the juvenile-onset form, whereas those with two A-type mutations generally manifest adult MLD (2).

The MLD-Mouse Model. Many inherited diseases lack a naturally occurring animal model. MLD has not been observed in animals other than humans which hampers studies of its molecular pathogenesis. The link between lipid storage and demyelination on the molecular level is unknown. An animal model would facilitate studies on the pathogenesis and the effects of different gene therapy approaches to this disease. To this end, an ASA-deficient mouse was generated (7).

The ASA gene was disrupted by cloning the neomycin resistance gene (Neo) into exon 4 (7). Cell and biochemical analysis of the homozygous mutant has revealed findings similar to MLD—i.e. deficiency in ASA mRNA and enzyme activity, and lipid storage in fibroblasts, CNS oligodendrocytes, and peripheral nerves. A focus of sulfatide storage and demyelination has been detected in the acoustic ganglion, and the mice are deaf. The animals have some inability to perform in the turning rod test, indicating a deficit in motor coordination and involvement of other areas of the brain. Furthermore, examination of the brain indicates that the Purkinje cell layer is abnormal (7).

The storage pattern is comparable to that of affected human, but gross defects of white matter were not observed up to the age of 2 years. This situation is reminiscent of the studies of homologous recombination and "knockout" of the mouse hypoxanthine-guanine phosphoribosyltransferase gene (HGPRT). In the HGPRT mutant mouse, alternative metabolic pathways exist which are sufficient to compensate for the loss of a gene product. It was subsequently reported that when inhibitors of purine metabolism were administered, these mice exhibited abnormal grooming and self-mutilative behavior mimicking the Lesch-Nyan syndrome (8). Such alternate biochemical pathways may exist in the CNS of the MLD mouse which prevents the severe clinical pathology observed in humans. Hence, it may be important to examine methods to induce the MLD phenotype in the mutant mouse. Because of its unique phenotype and aberrant biochemistry, this model will serve as a tool to better understand the differences between mouse and human sulfolipid metabolism, and thus provide valuable insight concerning the pathogenesis and pathophysiology of MLD.

Bone Marrow Transplantation. Bone marrow (BM) derived cells are able to migrate and gain entry to the developing and adult brain (9,10). In fact, gene marked bone marrow progeny have been shown to contribute to the microglia and macrophages in the CNS (11). Thus, it has been suggested that, 1) bone marrow transplantation (BMT) may provide a means of delivery of enzyme-competent donor cells to the CNS and/or 2) degradation of CNS storage material may occur by secretion of enzyme from donor microglia (derived from the transplanted bone marrow) and re-uptake by resident storage cells (cross correction). BMT has been carried out in several human lysosomal storage diseases including MLD, and while normalized levels of enzymatic activity are typically observed in peripheral blood leukocytes after successful BMT, reports have varied concerning improvements in the neuropsychological status of patients with CNS involvement (12–14). Others have reported that BMT has resulted in clinical improvement in a limited number of individuals with juvenile MLD (15,16), however no improvement has been noted with BMT in infantile or late infantile type MLD (16). Another example of the variability of BMT in the diseases involving CNS is the MPSVII mouse model. Retrovirus-mediated gene transfer and transplantation of BM in the MPSVII mouse provided evidence that genetically-cor-

rected cells reverse the visceral storage in the MPSVII mouse (17,18) while the brain remained unchanged in these mice, detection of enzyme in brain was equivocal, and no improvement in the behavioral abnormalities was observed (19,20). Conversely there were positive CNS changes in the α-mannosidosis cat model after bone marrow transplant (21). Although different cats transplanted at different ages still showed some skeletal deformities, BMT resulted in an α-mannosidase activity of 9–40% of normal in the CNS. This explains the absence of storage vacuoles in neurons and most other cell types in the cerebral cortex, except some microglia scattered throughout the brain, as compared to the untreated control. Treated animals showed an entirely normal thalamus, caudate, brain stem, and spinal cord. There was a mild loss of Purkinje cells and minimal neuroaxonal dystrophy as compared to the untreated cats (21). The most likely explanation for this finding is that α-mannosidase present in the donor BM derived cells is available to and can enter cells of the CNS after transplantation. The exact mechanism allowing the transfer of α-mannosidase from donor-derived cells to neurons of the CNS following BMT is unknown. It might be explained by the fact that cells of hematogenous origin (monocytes) invade the brain and establish brain macrophages or microglia that secret the normal enzyme in a form available to neurons and other diseased cells in CNS. Other BMT studies using canine models of fucosidosis (22), mucopolysaccharidosis type I (23), and murine mucopolysaccharidosis type VII (24) demonstrated enzyme activity in brain ranging from 1% to 20% of normal, concomitant with reduced amounts of intraneural storage. These findings provide evidence that BMT can result in transfer of lysosomal hydrolases into the CNS.

However the threshold of enzyme activity sufficient to reverse or prevent the disease especially in the CNS may be different between various lysosomal diseases. Recent studies attempting to understand the late onset, chronic forms of lysosomal β-hexosaminidase A and arylsulfatase A deficiencies, using substrate-fed fibroblasts in culture, have indicated that this threshold can be as low as 10-15% of normal (25). Another important factor in the BMT is the age of the recipient animal or the stage of development of the CNS (26). There has been one report of BMT in a child with α-mannosidosis who died 18 weeks after successful engraftment (27). This child showed evidence of enzyme correction in liver and spleen, but only very low levels of normal α-mannosidase activity was found in brain, with no evidence of decreased neuronal vacuolation. The genetic and epigenetic factors that make some lysosomal diseases more amenable to BMT than others are unknown.

Thus, while retroviral transduction and transplantation of hematopoietic cells may soon prove useful clinically for diseases of the viscera (Gaucher disease) and immune system (ADA deficiency), attempts to use HSC alone to target the CNS in lysosomal storage diseases with neuropathology (such as MLD) may be more difficult. How many BM cells can gain access to the CNS following BMT, and whether these cells provide sufficient cross-correction of enzyme in such diseases, are but a few of many unresolved questions.

Postnatal BMT may not prove to be the perfect therapeutic treatment for some diseases. If it were performed at a very early age or antenatally when the formation of the BBB is not fully complete, and when the brain is still in the process of myelination, and there is a greater chance that BMT may be efficacious for diseases such as MLD.

Retroviral Gene Transfer. The principle of gene therapy is to achieve endogenous production and/or secretion of a gene product by delivery of a gene to a population of cells. Gene delivery vehicles for gene therapy purposes can be broadly classified as either viral or nonviral. Here, we will only discuss the viral delivery systems proposed for this project. There are two important criteria which influence the choice of vectors. First, the accessibility of the target tissue to be manipulated dictates whether host cell transduction can be performed in vivo or ex-vivo and, secondly, whether persistence of transgene expression is to be sustained or temporary. The most widely used gene transfer delivery system is the murine leukemia retroviruses (MLV). The MLVs and their life cycle have been well studied and characterized (28).

Because of its accessibility and medical significance, the bone marrow (BM) has become a desired target for gene transfer. Previous work in our laboratory and others has shown that hematopoietic stem cells (HSC) isolated from the bone marrow can be readily transduced with retroviral vectors with high transduction efficiencies (TE) (29–31). Following transplantation, complete repopulation of BM by transduced HSC was observed. BM-derived cells which expressed the transgene were found in the viscera and blood throughout the lifetime of these transplanted animals (29,32) Krall et al. have reported that in the brains of mice transplanted with retrovirally transduced BM, 20% of microglia were positive for the presence of a transgene 3 to 4 months post-BMT (11). Retroviral vectors have also been shown to transduce and express a transgene in purified human $CD34^+$ progenitors isolated from BM, cord blood (CB), and mobilized peripheral blood (PB) (33–35). Dunbar and ourselves have shown an average TE of between 20 to 30% in these cells. In a clinical trial of breast cancer

patients (36), CD34+ cells from the PB or BM were transduced and reinfused into ablated patients. Four of the five patients analyzed had evidence of marked cells in their marrow or peripheral blood (<1%) at the time of engraftment. In an ongoing Phase I clinical trial in our laboratory, CD34+ enriched cells from the PB of Gaucher patients are retrovirally transduced with centrifugal enhancement and then transplanted without preparative myelablation (37). To date, all three study subjects have demonstrated the presence of PCR positive cells in their PB and BM at levels between 0.1 and 1%. Additionally, a quantifiable increase in the specific enzymatic activity of PB leukocytes of all three subjects has been observed. These results suggest that CD34+ progenitors can be transduced and transplanted into patients with subsequent engraftment and expression at levels which may be clinically relevant.

Oligodendrocyte Transplantation. An alternative approach to the treatment of demyelinating diseases may be oligodendrocyte cell transplantation. This strategy offers the combined possibility of delivering a larger number of enzyme competent cells to the CNS as well as supplying cells capable of remyelinating damaged areas of the brain. Early experiments with mouse models of Parkinson disease (PD) suggested that transplantation of fetal tissue into the brain resulted in survival of grafted cells, synthesis and release of dopamine, and phenotypic reversal of abnormal behaviors (38). Fetal brain tissue has also been used in various human clinical trials. The first human fetal brain tissue transplants were done in 1987 using the ventral mesencephalon (VM). Madrazo et al. showed that treatment of 4 PD patients with fetal VM tissue resulted in significant amelioration of rigidity, bradykinesia, postural imbalance, gait disturbance, and facial expression, as well as an increased sensitivity to L-dopa medication (39–41). Lindvall et al. also used fetal VM to treat 4 PD patients and obtained similar results. Although improvements have been observed to a varying degree in almost all PD patients subjected to neural grafting, no complete reversal of multiple or single parkinsonian symptoms has been obtained even in the best cases (42–46). These findings suggest that single localized transplants may not be sufficient to correct diseases with extensive CNS pathology.

Global disorders of the brain will likely require repeated transplants at multiple sites to arrest or reverse disease pathology. Transplantation of newborn or fetal tissue into the CNS of animals deficient in myelin production, shiverer mouse (shi) and myelin deficient rat (md), has demonstrated that oligodendrocytes from within the grafted tissue can produce myelin and are capable of migrating several centimeters from the site of transplantation into the recipient brain or spinal cord (47,48). Success in grafting tissue in the brains of these animals is perhaps due in part to the relative "immune-privileged" status of the brain. As suggested by BMT studies, the diapedesis of immune cells from the blood to the brain takes time and the number of the hematogenous cells that gain access to the brain is limited. This could result in a reduced ability of the host immune system to eliminate the donor tissue or cells (49–51). While the blood brain barrier (BBB) is a major obstacle in intravenous enzyme therapy or BMT, it can be circumvented by direct transplantation to the brain.

The establishment of oligodendrocyte progenitor (OP) cell cultures isolated from fetal or newborn brain has added greatly to the understanding of the developmental cell biology of glial cells (52,53). Of these cells, the oligodendrocyte-type2 astrocyte (O-2A) cell, originally derived from the optic nerve of newborn rats, has been studied in most detail. O-2A has a bipolar morphology, and upon differentiation undergoes a dramatic change in morphology which is coincident, immunohistochemically, with the expression of type 2 astrocyte markers or oligodendrocyte markers. In vitro, the cells cease to divide in absence of serum or cytokines and begin to express galactocerebroside, suggesting that differentiation into oligodendrocytes is the constitutive pathway (54). The addition of 10% FBS induces expression of glial fibrillary acidic protein (GFAP), and most cells differentiate into type 2 astrocytes (55). Coculture of O-2A cells with type 1 astrocytes, or their conditioned media, resulted in several rounds of division prior to differentiation suggesting that type I astrocytes may supply growth factor(s) in vivo (56–59). Other cell types and cytokines have been implicated in the control and differentiation of OP cells (60–63).

The isolation of bipotential glial cell progenitors and the development of methods of maintaining them in culture has prompted transplantation studies using these purified cells. Transplanted oligodendrocytes were shown to be capable of limited migration, and that the migration pathways of the transplanted cells correlated with the expression of specific markers at the sites of demyelination (64–67). A cell line (CG-4) derived from the O-2A cell line was transplanted into the spinal cord of the myelin deficient rat and shown to be capable of survival, migration, and remyelination (68,69). Baron-Van Evercooren et al. recently established that a Schwann cell line transplanted into the CNS could engraft and compete with host oligodendrocytes to remyelinate denuded axons of the spinal cord. The myelin produced from these cells was structurally normal and could restore nerve conduction. However, more recently,

Avellana-Adalid et al. (70) was able to expand primary cultures of OP from newborn rats into large numbers of homotypic aggregates termed oligospheres. Contrary to the CG4 cell line, these oligospheres could proliferate for over a year and their progeny conserves its bipotentiality in vitro and their capacity to form myelin after transplantation into CNS. Similarly, Snyder, et al., have reported the development of a mouse neural progenitor cell (C. 17) which can contribute to neuronal and glial lineages following transplantation into the brain (71). When transplanted into the brain of the mucopolysaccharidosis VII mouse, the authors reported a partial reduction of lysosomal storage in the host brain, presumably by the exchange of enzyme (glucuronidase) with donor cells (72).

These results support the use of OP cells in studies as a source of transplantable cells for the repair of demyelinated areas of the CNS and possible cross correction of enzymatic deficiency. Transplantation of CG4 cells or oligospheres directly into the brain of the MLD mouse model may provide answers about the ability of the oligodendrocyte progenitors to repair brain damage in MLD patients.

Adeno-Associated Virus Vectors. Although retroviral vectors are the best characterized gene transfer system, the requirement of active cell division for integration into the target cell leads one to consider alternate vectors for use in the brain. One such vector is the recombinant adeno-associated virus (rAAV).

The AAV genome is approximately 4.7kb long and contains two open reading frames (ORF), flanked by two inverted terminal repeats (ITR). The ORFs code for two families of proteins. The first ORF codes for the proteins responsible for viral replication (Rep78, Rep68, Rep52, Rep40), while the second codes for the proteins (VP1-3) responsible for viral encapsidation. Each ITR is 145 base pairs long and forms a T-shaped hairpin structure which is used to initiate DNA replication (73). The sequences necessary to package the viral DNA into virions are contained in the ITRs. The life cycle of AAV is either lytic or latent depending upon whether or not the cell is coinfected with helper virus. In the absence of helper virus, wild type AAV integrates into the host cells genome and is carried as a provirus. When an AAV infected cell, integrated or not, is coinfected with helper virus, a lytic propagation cycle is induced. AAV has a broad host range and infects most mammalian cells, even those which are quiescent. These properties support the use of rAAV as an appropriate vector for gene transfer studies into the CNS.

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector. Since the only required cis acting sequences necessary for packaging the viral DNA into virions are contained in the ITRs, the entire region between the ITRs can be deleted and replaced with the gene of interest (73). While this carrying capacity may prevent rAAV from delivering larger genes, it is sufficient for the delivery of smaller genes or antisense cDNA. The second characteristic that makes AAV a good vector candidate is its safety. AAV is not pathogenic and has not been associated with any disease. Since AAV is naturally replication defective, generation of a replication competent virus from cells transduced with rAAV would require that the cell become infected simultaneously with wild type AAV and helper virus. The possibility that this may occur is small. Finally, the removal of all viral coding sequences in the production of rAAV minimizes potential immune reactions against viral proteins, thereby significantly lowering the risk of an inflammatory response (73).

Upon infection of a human cell, wtAAV preferentially integrates into a specific region of chromosome 19 (74-78). In the absence of Rep, AAV appears to integrate at random (79). Recent evidence has established that rAAV can also be expressed as an episome. The ability of rAAV to integrate may be dependent upon the multiplicity of infection (MOI) and appears to occur only at higher MOIs (74). Hargrove et al. established that the differences in MOIs required to infect cells is dependent on the number of receptors expressed at the cell surface (74). Once the virus is in the cytoplasm of the host cell, the single stranded rAAV genome must convert into a double-stranded transcriptional template (80,81). Alternately, formation of double stranded DNA could occur by annealing once the genome is uncoated, since both the positive and the negative viral DNA strands are packaged, but in separate virions. As reported by Hargrove, such annealing would be enhanced at higher MOIs due to the increased probability that the cell would contain multiple copies of the two single-stranded forms of the virus. Also the amount of double-stranded template may be relevant to the frequency of genome integration. Trace contaminants of adenovirus or AAV Rep proteins, even in highly purified rAAV preparations, could influence the behavior of the vectors by facilitating viral uptake or influencing the final destination of the rAAV genome within the cell.

The limitation of AAV is its production. Although it can be purified and concentrated, which are advantages, it also has to be rendered free of adenovirus, and therefore production is more complicated than for other vectors. In addition, it is difficult to produce AAV at large scales and to high titer. This issue has been the

driving force for much research. Recently Vincent et. al. looked at the limiting component(s) required for rAAV packaging in order to improve the titer of rAAV (82). It has been shown that rAAV is not limited by the level of replicated viral DNA or the rep protein but is limited by capsid protein production. The rAAV yield was improved approximately 10-fold when cap gene expression was increased. Modification of the protocol of transfection using plasmid DNA complexed to replication-competent Adeno virus modified with polylysine has also been reported to result in a 40- to 240-fold increase in rAAV packaging (82) over the standard calcium phosphate method (83). The selection of the producer cell line used may also be a limiting factor for rAAV production. COS cells could be more readily transfected with the vector and helper plasmids than 293 cells when a calcium phosphate transfection strategy was used (84). Also using a helper plasmid containing the SV40 replicon gave a packaging efficiency of over 103 rAAV particles/cell (84). To avoid the inefficient transduction step, Clark et al. constructed a cell line that contain both the vector and AAV rep/cap genes which allows production of rAAV by Ad infection alone. With this cell line, the yield was 400 particles/cell (85).

The most fruitful application of rAAV may be in the transduction of brain. AAV is unique in its ability to infect and stably integrate in non-dividing tissue. AAV vectors have been successfully used in gene transfer studies in the CNS. Kaplitt et al. found that AAV could transfer E. coli β-galactosidase (lacZ) gene, or the tyrosine hydroxylase gene, and sustain expression of the gene product in the CNS with no obvious signs of neurotoxicity. More importantly, for up to 3 months, expression of tyrosine hydroxylase within the caudate nucleus partially ameliorated the motor deficits (86). These studies established the ability of AAV-mediated gene transfer to ameliorate a degenerative CNS process and have prompted us to examine rAAV as vector for delivery of the ASA gene into the CNS as a potential treatment for MLD.

Antenatal Gene Transfer

Neurophysiological Effects Of Storage Disorders in the Fetus. Development of the brain is a complex process which begins with neural tube formation and continues until after birth. The events that follow the development of the external form include proliferation of neurons and glia, migration to specific sites, organization into intricate circuitry and finally myelination. These events span a period from the second month of gestation to adult life. In many neurodegenerative storage disorders with clinical onset during infancy, there is evidence to suggest that the abnormal CNS storage of the substrate begins in utero. An increased concentration of sulfatide has been shown in the cerebellum, brain stem and spinal cord of a 24 week old fetus with MLD (82). Excess of sulfatide has also been noted in myelin isolated from another fetus with MLD (89). In a series of electron microscopy studies in fetuses (gestational age 12 to 22 weeks) with Tay Sachs disease, Adachi et al found membranous inclusions in the anterior horn cells, spinal ganglia, retina and pituitary gland (88). In neuronopathic Gaucher disease too, available evidence leads one to conclude that glucosylceramide accumulation in brain produces dysfunction in surrounding cells long before discrete pathologic changes are seen (90). Advanced pathological alterations in fetal tissues including the CNS have been documented in Gaucher disease (91,92). A subset of type 2 or acute neuronopathic Gaucher has been described with onset of severe disease in the fetus (93). It is likely that these diseases not only cause degeneration of preformed neurons but also interfere with normal developmental events with potentially irreversible consequences. Neurodevelopmental outcomes in these patients will therefore be greatly improved by early treatment in the fetus provided it is safe and effective.

Fetal Liver as a Source of HSC. Fetal liver is a potential source of HSC for purposes of transplantation. Hematopoiesis develops in an essentially identical process in all mammalian species. It begins in the yolk sac, transfers to the liver and spleen, and initiates in the bone marrow towards the end of gestation (94). Liver is the principal site of hematopoiesis between 7–19 weeks of gestation in humans (94), 11–16 days in mice (95), and 24–60 days in sheep (95).

Fetal liver cells have the capacity to differentiate into a complete repertoire of erythroid, myeloid and lymphoid cell lineages (97). The concentration of committed progenitors and CFUs is lower than in the marrow. Fetal liver cells form only one third of the CFUs seen when an equal number of nucleated bone marrow cells are injected into lethally irradiated mice (98). On the other hand, fetal liver has a larger proportion of self-renewing, pluripotent stem cells, a significant fraction of which are likely to be replicating and amenable to transduction with retroviral vectors. This observation is supported by the gene transfer experiments comparing the efficiency of retrovirus mediated transfer of the Neo-R gene into fetal and adult hematopoietic cells. Both human and bovine fetal cells exhibited a significantly higher degree of Neo-R transfer than adult hematopoietic cells (99).

Mouse fetal liver contains very few, if any, immunocompetent cells (100). However, in larger animals

and in humans the fetus is capable of an immune response (95). In humans, lymphocytes can be detected in the fetal liver as early as 7 weeks of gestation but comprise only 1% of the hematopoietic compartment and are immunologically immature (96). By 12 weeks of gestation, mitogen responsive lymphocytes are found in the thymus. Alloreactive T-cells are present in the blood and organs by 14–16 weeks of gestation. Transplants of allogeneic fetal liver 14 weeks or less in gestation have not been associated with severe GVHD (94,95). Thus, fetal liver at 8-14 weeks in humans, 11–13 days in mice, and 30–60 days in sheep should be an ideal source of actively replicating HSC and progenitors useful for studying transplantation and genetic correction.

Models may be useful for the assessment of transduction efficiency and expression of the human GC gene in human cells in vivo. The severe combined immunodeficient mouse (SCID) has recently been used to study human hematopoiesis (101). In this model 50% or more of the bone marrow of the mouse is of human origin and persists for several months. Retroviral infection in vitro of human bone marrow or enriched stem cell preparations followed by their transplantation into the SCID mouse has permitted the assessment of the efficiency of transduction and persistence of expression of transferred genes in human hematopoietic cells in vivo. This model system may be useful in evaluating these parameters in studies of the transfer of the GC gene to human hematopoietic cells. Transplantation into the pre-immune fetus may be an equivalent to the SCID mouse model and would be useful in studying HSC transplantation and gene transfer. Further, transgenic models of human genetic disease have been and will be useful in studying the potential for gene therapy.

Gene Transfer to Fetal HSC: Role in Prenatal Stem Cell Therapy. The pre-immune fetal host is uniquely tolerant of allogeneic fetal tissue grafts. Fetal tolerance resulting in permanent Chimerism has been documented to occur naturally in animal and human twins with shared placental circulation (102,103) and has been experimentally demonstrated in several animal models (104-106). Human clinical experience is, however, limited. Touraine et. al. reported on 5 fetuses transplanted prenatally at 12 to 28 weeks of gestation with fetal liver cells. Three had immunodeficiency disorders and two had thalassemia major. Three survived to term with evidence of engraftment and clinical improvement (107). Even if immunological disparity is not a limitation to allogeneic stem cell transplantation, in utero transplantation of genetically modified fetal HSC has at least two foreseeable uses: (1) In the possible event of a delay in diagnosis to a time when the fetus less immune tolerant (after 16 weeks of gestation), autologous transplantation of HSC after genetic correction would be superior to an allogeneic HSC graft. In this regard, studies of in utero gene transfer in allogeneic or syngeneic models provide information pertinent to autologous transplantation. (2) The use of in utero HSC transplantation, at the present time, is limited by low grafting efficiency (1–10%). With current gene transfer techniques, it is possible to achieve enzyme expression several times above normal (29). This may be beneficial by permitting a small number of engrafted cells to achieve a therapeutic effect by secreting the gene product into the circulation which is taken up by cells throughout the body (19,108,109).

The studies cited above suggest a possible role for gene transfer and cellular transplantation in the treatment of lysosomal storage diseases with CNS involvement, such as MLD. Several possible mechanisms may exist which could make such a strategy efficacious: 1) Degradation of storage material may occur by genetically-corrected enzyme-competent cells delivered directly to the brain, or cells derived from transduced cells (BM, transduced brain cells, oligospheres) thereby reducing the possibility of accumulation of the storage material in brain. 2) The transgene product (enzyme) may be secreted from donor glial cells or other cells to neighboring storage cells 3) spread of transduced cells, oligospheres or other brain cells.

The ability to reverse or prevent these cell biologic and biochemical changes by gene transfer and cell transplantation would be powerful data supporting the development of this approach as a therapeutic modality for MLD. Some of these experiments are proposed below. Many important studies of gene transfer and transplantation are now possible given the development of 1) an animal model of MLD, and 2) vectors which efficiently transduce transplantable cells such as oligodendrocytes and oligospheres.

Early Results and Studies in Progress

Retroviral Gene Transfer to HSC. Previous work in our lab has shown that a retroviral vector (MFG-GC) that incorporates the glucocerebrosidase cDNA (GC) can transduce and express GC in rodent bone marrow and human $CD34^+$ bone marrow progenitors (33).

Ecotropic and amphotropic retrovirus producing cell lines (VPC) of the MFG-ASA vector, as well as an unreported "safety-modified" retroviral vector (R-ASA) have also been developed and tested in our laboratory. These vectors incorporate the human ASA cDNA. R-ASA includes a SacII linker which creates a frameshift

in the reading frame of the wild type retroviral gag gene, preventing synthesis of this viral polypeptide in transduced cells. A retroviral vector containing the β-galactosidase gene (MFG-LacZ) has also been used in our laboratory. In each case, the ASA or LacZ transgene has been inserted into the retroviral genome at the transcription start site of the deleted retroviral env gene and transcription is driven by the endogenous 5' LTR. VPC were obtained by co-transfection of MFG-ASA or R-ASA with a selectable (pSV2Neo) plasmid into ecotropic and amphotropic packaging cell lines, psi-cre and PA317 respectively (110,111).

Infectious cell supernatants from cell clones were screened to identify high titer VPC by examining enzymatic activity in 3T3 fibroblast target cells. A variety of cultured cells, including human MLD patient fibroblasts and human $CD34^+$ cells, were exposed to these infectious supernatants and subsequently shown to express ASA activity far above that in non-transduced cultures (112).

We have also shown that transduction of murine bone marrow with MFG-ASA resulted in highly efficient gene transfer to hematopoietic progenitor cells. This was demonstrated by the presence of vector DNA in 90% of spleen colonies 12 days after gene transfer and BMT. The vector sequence was detected in macrophage cultures derived from transduced donor BM cells, and subsequently in the BM, spleen, lung, liver, and brain of long term reconstituted mice (4 months after BMT), indicating that BM-derived cells had migrated to and repopulated the viscera and the brain. Furthermore, the DNA signal was present in 100% of the spleen colonies of mice transplanted secondarily with bone marrow from long term reconstituted mice, indicating not only successful reconstitution, but evidence for transduction of the pluripotent HSC in the primary recipients. Enzyme activity in the brains of mice 4 months after BMT was unchanged as compared to non-transduced control samples, probably as a result of the small number of BM-derived cells that enter the brain. However, approximately 2 out of 3 spleen colonies obtained from secondarily transplanted mice expressed arylsulfatase A above control levels, indicating that the MFG vector was continuing to express the transgene (113).

The in vivo results obtained in these studies of MFG-ASA are nearly identical to those obtained with MFG-GC. Thus transduction and expression of a transgene in this system are not specific to the transferred gene, but rather are features of the vector. The simplified design of the MFG construct, use of the wild type promoter within the 5' long terminal repeat sequence (LTR), and inclusion of splice donor/acceptor sites are believed to be important for expression of the transgene from this vector.

Transduction and Transplantation of CG4 Cells. Given the relatively long periods of time required for diapedesis to the CNS, it is likely that the blood-brain barrier (BBB) significantly limits such movement of BM-derived cells following BMT. This is of particular concern in the adult animal, where turnover of microglial cells may be slow and the BBB is fully developed.

Hence, alternate strategies to deliver genetically modified cells to the brain are being considered. One strategy would be to transplant hematopoietic cells into the fetus, or newborn animal, during a developmental period of active myelin restructuring, and prior to the formation of the adult BBB. Studies of the MPSVII mouse suggest that early therapeutic intervention provides a better clinical response (17,18). A second approach involves transduction and direct injection of an oligodendrocyte precursor cells into the CNS. The latter approach would circumvent the BBB, and also allow experimentation in adult animals.

A rat oligodendroglial progenitor cell line (CG4) has been identified which is cytokine dependent and has the potential to differentiate into oligodendrocyte or type 2 astrocyte cell types. Immunocytochemical studies have shown that CG4 cells are bipolar and upon differentiation, they gain characteristics of astrocytes or oligodendrocytes. We have used antibodies for the immunohistochemical detection of the oligodendrocyte cell surface marker 01 and the astrocyte marker glial fibrillary protein (GFAP).

CG4 cells were cultured in poly-ornithine-coated tissue culture dishes with the addition of platelet-derived growth factor (PDGF) and fibroblast growth factor (BFGF) from the conditioned media of the neuroblastoma cell line B104, as previously described (62). Upon withdrawal of the B104 conditioned media, the cells stopped dividing, gained cell processes characteristic of oligodendrocytes, and stained positively with the 01 antibody. The addition of 10% fetal bovine serum induced CG4 cells to take on a more stellate morphology and express GFAP. CG4 cells were transduced by the MFG-LacZ vector under a centrifugal force of 2400 times gravity. Under these conditions, retroviral transduction is enhanced approximately 5-fold and approximately 95% of CG4 cells expressed the LacZ transgene 2 days after transduction, as detected by histochemical staining with X-gal. Transduced cells were passaged and maintained in culture for >3 weeks, and LacZ expression was detected throughout this period in a majority of the cells, indicating that transgene expression is long lived.

CG4 cells were transduced with MFG-LacZ (as above) and, 24 hours later, transplanted into the brains of Sprague-Dawley rats by stereotaxic injection in the corpus callosum or neostriatum. After 2, or 7 days post-transplantation, the rats were sacrificed, and coronal sections of their brains were examined immunohistochemically with the use of an antibody specific for β-galactosidase. CG4 cells expressing the LacZ transgene were observed to have migrated several millimeters away from the needle tract in each group of animals. Migration of cells was especially evident in the corpus collosum where the donor cells moved laterally (in the same plane as the section), presumably along axonal fiber tracts (unpublished data). LacZ-positive donor cells had morphologies which resembled both bipolar precursors and multi-process (oligodendroglial) cell types.

In a recipient that received transduced CG4 and survived 28 days, weak staining of $LacZ^+$ donor cells was detectable along the needle tract. However, the donor cells had lost their glial morphology and appeared rounded and granulated at this time point.

This data indicates that CG4 cells can 1) be transduced by retroviral vectors, 2) express a retroviral transgene for at least 21 days in vitro and 7–28 days in vivo, 3) be transplanted into rat brain and survive for at least 7 days in vivo, and have some migratory potential in the rat brain.

Adeno-Associated Virus Vectors. A rAAV plasmid vector pJJ-3ASA was developed by Dr. Jing Fang Wei in our laboratory. The strategy for plasmid PJJ-3ASA construction has been described elsewhere (114).

The rAAV successfully infected murine and human patient fibroblasts as demonstrated by enzymatic and DNA analysis of infected cell clones. ASA enzyme activity of AAV-ASA infected 3T3 cells was 4 to 9 fold higher than in non-infected controls. In infected MLD patient fibroblasts, most of the clones had enzymatic activities that exceeded that of a normal cell line (0637D). Some clones had very high activities, up to 500 times the activity of the disease control. Southern blotting results showed that on average, the vector integrated 1–2 copies of pJJ-3ASA in the target cell genome (114).

This data demonstrates that rAAV vectors can be used for introducing the ASA gene into murine and patient primary fibroblasts. The amount of enzyme expressed in the transduced cells exceeded the amount normally found in cells and persisted for as long as the cells were carried in culture (>10 weeks). These studies support the possibility of using rAAV vectors in studies of ASA gene direct transfer to the brain of rats and mice.

Antenatal Gene Transfer. The retroviral vector (MFG-GC) was used to perform these preliminary studies. This is the same vector used in bone marrow transplantation studies. It is useful too demonstrate the principle of gene transfer to FLC. The MFG vector is based on the MoMuLV (Moloney Murine Leukemia Virus), in which the env gene has been replaced by the human GC cDNA. The construction of the vectors has been described previously.

After transduction by conventional incubation, about 10–15% of the fetal liver mononuclear cells expressed the immunoreactive GC protein and stained positive with mAb 8E4. The control cells infected with MFG-lacZ were consistently negative for the human GC protein. Cells were also analyzed for glucocerebrosidase (GC) enzyme activity as a functional assay for the transferred human GC gene.

By contrast when centrifugation was used for viral inoculation, 3–4 times as many cells stained positive for the human GC protein. Of the fetal liver cells (FLC) transduced with MFG-GC, 60% of the cells stained positive. The MFG-lacZ infected controls were negative. MFG-GC transduced FLC infected without centrifugation had a GC activity of 331–509 Units/mg of protein, which is 2–3 fold the activity in control cells (160 units/mg). MFG-GC transduced FLC infected with centrifugation had a GC activity of 1572–1605 Units/mg of protein, which was 8–9 times above controls (166 U/mg).

In Utero Transplantation with MFG-GC Transduced Fetal Liver Cells. In one experiment, 15 C57bl/6J fetal mice at D-13 of gestation were transplanted by intraplacental injections of MFG-GC transduced fetal liver cells. $5–10 \times 10^5$ cells suspended in 5μl volume were injected into each fetus. Nine out of the fifteen (66%) fetuses were born at term. The neonates survived to adulthood (3 months) with no obvious adverse effects from fetal intervention. The animals were sacrificed at 3 months and tissues (liver, spleen, bone marrow and brain) were analyzed for carriage and expression of the transferred human glucocerebrosidase gene. All of the nine animals showed the presence of the transgene in liver, spleen, and bone marrow by PCR analysis.

To estimate the frequency of transduced donor cells in the bone marrow, DNA from the bone marrow samples was progressively diluted with negative control 3T3 DNA, such that marrow DNA ranged from 1.5 μg to 0.001 ng with total DNA being constant at 1.5μg. The transferred human GC gene sequences were amplified using nested primers and all samples were analyzed twice. A positive band in both reactions indicated the presence of at least one copy of the vector or one transduced donor cell. The lowest dilution with a positive band was used to estimate the number of total cells

which contained a minimum of one transduced donor cell. Results on two of the animals, #M05 and #F06 are discussed. In M05 one transduced donor cell is present in 0.1ng DNA (150 diploid cells) which is equivalent to an estimated frequency of 1/150 cells. In F06 this amounts to 1 in 0.1ng or 1/15 cells.

Bone marrow and liver from the nine animals were analyzed by RT-PCR. All nine animals demonstrated the presence of viral transcripts of the human GC gene in their marrow and seven of the nine in their liver, 13.5 weeks after transplantation in utero.

Brain tissue from the nine animals transplanted in utero was analyzed at twelve weeks of age or 13.5 weeks post-transplantation. Vector (MFG-GC) specific primers (AB1/2) were used in the PCR assay to identify transgene bearing donor cells or their progeny. Eight of the nine animals were positive for the transgene.

Total cellular RNA from the brains of 12 weeks old mice transplanted in utero was analyzed by RT-PCR for persistence of transgene expression. RT-PCR done in the absence of RT (reverse transcriptase) was consistently negative excluding contamination with DNA. Eight of the nine animals were positive for expression of the transferred gene.

Summation. The approaches and results presented in this paper indicate that gene transfer as a therapy for lysosomal storage disorders requires a significant amount of laboratory and clinical investigation. A variety of studies employing several different systems will be necessary to decide which of the approaches will be effective for these disorders, each of which has its own unique characteristics and complications.

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Klinische Pharmakologie (1994), 11(Drug Interaction Studies during Drug Development), 38-42.

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Gene therapy for lysosomal storage disorders

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The lysosomal storage disorders (LSD) are monogenic inborn errors of metabolism with heterogeneous pathophysiology and clinical manifestations. In the last decades, these disorders have been models for the development of molecular and cellular therapies for inherited metabolic diseases. Studies in preclinical *in vitro* systems and animal models have allowed the successful development of bone marrow transplantation (BMT) and enzyme replacement therapy (ERT) as therapeutic options for several LSDs. However, BMT is limited by poor donor availability and high morbidity and mortality, and ERT is not a life-long cure. Moreover, the neuropathology present in many LSDs responded poorly, if at all, to these treatments. Therefore, gene therapy is an attractive therapeutic alternative. Gene therapy strategies for LSDs have employed *ex vivo* gene transduction of cellular targets with subsequent transplantation of the enzymatically corrected cells, or direct *in vivo* delivery of the viral vectors. Oncoretroviral vectors and more recently adeno associated vectors (AAV) and lentiviral vectors have been extensively tested, with some success. This review summarises the main gene therapy strategies which have been employed or are under development for both non-neurological and neuronopathic LSDs. Some of the *in vitro* and *in vivo* preclinical studies presented herein have provided the rationale for a gene therapy clinical trial for Gaucher disease Type I.

Keywords: animal models, BMT, enzyme replacement, gene transfer, lysosomal storage disorders, therapy

Expert Opin. Biol. Ther. (2001) 1(5):857-867

1. Introduction

The LSDs are a group of inherited disorders for which gene therapy offers a potential cure. The genes coding the lysosomal enzymes have been cloned and characterised and the gene products are therapeutic. As several laboratory studies and clinical experience have demonstrated, ERT reverses the visceral complications of lysosomal storage in several diseases, including Gaucher disease [1,2], Fabry disease [3-5], Pompe disease [6,7], Hurler-Scheie disease (mucopolysaccharidosis Type I, MPS I) [8] and Hunter disease (MPS II) [9]. Furthermore, allogeneic BMT studies have shown that engrafted haematopoietic cells reverse the signs and symptoms in some of the LSDs and may therefore be appropriate targets for gene transfer [10]. BMT may exert metabolic correction by allowing the repopulation of the recipient haematopoietic and lymphoid cells with normal donor-derived cells providing a self-renewing source of exogenous hydrolase. Metabolically normal repopulating cell types include mononuclear phagocytic cells, such as Kupffer cells and the microglia of the central nervous system (CNS), which are frequently involved in the pathobiology of LSDs. Moreover, BMT may provide correction by release of lysosomal enzyme from the donor cells to the deficient recipient cells. Intercellular transport may occur by

receptor-mediated endocytosis of secreted enzyme, or by direct transfer of enzyme by adjacent cells [11]. In developing gene therapy strategies for the LSDs, a primary concern is gene delivery to a target cell in which genetic correction is likely to be efficacious, stable and safe. At present, viral-mediated gene transfer is the only feasible approach which meets these requirements. However, different strategies will need to be taken to evaluate the potential of gene transfer as a therapeutic tool, due to the heterogenous pathophysiology of lysosomal disorders. In particular, the presence of CNS involvement in some diseases such as mucopolysaccharidosis (MPS) and metachromatic leukodystrophy (MLD) requires gene transfer strategies aimed at overcoming the limitations imposed by the blood-brain barrier (BBB).

2. Murine models of lysosomal diseases

Animal models of LSDs provide a powerful investigative tool, particularly when the animal phenotype closely matches the disease symptoms in humans. For example, the naturally-occurring MPS VII (*gus*$^{mps/mps}$) mice have been studied extensively and are found to have morphologic, genetic and biochemical characteristics which closely mimic those of human MPS VII (Sly disease) [12]. Affected mice have facial dysmorphism, growth retardation, deafness, behavioural deficits and shortened lifespan. Sly *et al.* recently produced a transgenic mouse expressing the human β-glucuronidase (GUSB) cDNA with an amino acid substitution at the active site nucleophile (E540A) and bred it onto the MPS VII (*gus*$^{mps/mps}$) background [13]. This new transgenic mouse is now tolerant to immune challenge with human GUSB and may therefore be useful for preclinical trials evaluating the effectiveness of enzyme and/or gene therapy with the human gene products likely to be administered to human patients with MPS VII. Another example of naturally-occurring murine model is the twitcher mutant for Krabbe disease [14].

For diseases not yet discovered in animals, the advent of gene targeting in pluripotent mouse embryonic stem cells has allowed the experimental development of murine models for most human LSDs. For example, the recently developed knockout mouse models for Fabry disease [15] and Pompe disease [16] have been useful in developing both enzyme and gene therapy strategies, even though the phenotype may not be similar to that of affected individuals. In some instances, the resulting phenotype is very significantly different from that observed in humans, due to alternative physiological pathways of lysosomal metabolism in rodents. An example is the transgenic glucocerebrosidase-deficient mouse model generated for Gaucher disease studies [17]. This mouse model exhibited a profoundly severe phenotype with perinatal lethality that precluded its use in most studies of gene transfer and cell transplantation. Similarly, an ASA-deficient mouse generated for MLD showed a lipid storage pattern in fibroblast, CNS oligodendrocytes and peripheral nerves reminiscent of the human MLD phenotype, but gross defects of the white matter were not observed up to the age of 2 years [18]. Alternate biochemical pathways may exist in the CNS of mice that prevent the severe clinical pathology observed in humans. Mice with a targeted knockout of the β-hexosaminidase A gene [19] offer another example of phenotype discrepancy between an animal model and its corresponding disease in humans.

3. Gene transfer for LSDs without prominent CNS involvement

3.1 Gaucher disease

Gaucher disease is the autosomally recessively inherited deficiency of the lysosomal enzyme glucocerebrosidase (GC), which catalyses the hydrolytic cleavage of glucose from glucocerebroside. The accumulation of glucocerebroside in the lysosomes of macrophages results in multisystem damage, including hepatosplenomegaly, gradual replacement of BM and skeletal deterioration [20]. In most cases of Gaucher disease, there is no evidence of neuropathology (Type 1 variant). The characteristics of the pathobiology of Gaucher disease make it a good candidate for treatment strategies based on correcting enzyme deficiency in macrophages by BMT, enzyme replacement and gene transfer. In Gaucher patients who received allogeneic BMT, the organomegaly resolved gradually, Gaucher cells disappeared from the BM and the clinical status improved [21-23]. A commercial preparation of mannose-terminated GC known as Ceredase™ (Imiglucerase) is now widely used as enzyme replacement therapy in patients with Gaucher disease and effectively reverses the visceral manifestations of the disease [1,20,24,25]. The success of BMT and the possibility of correcting the phenotype of Gaucher disease by the administration of the glucocerebrosidase gene product have shown that enzymatic correction of only one cell type - the macrophage - results in effective therapy. The fact that BMT is effective provides a rationale for somatic cell gene therapy strategies aimed at transducing autologous BM stem cells. Furthermore, because expression of the GC gene in the macrophage lineage alone may be sufficient for therapy, one could consider, as less permanent alternatives, gene transfer to committed macrophage precursors, peripheral blood monocytes, or to cultures of somatic cells which may secrete the enzyme in the circulation for macrophage uptake. Success in long-term expression of the GC gene in mouse haematopoietic cells has been demonstrated [26]. The authors' study has shown that murine whole BM could be transduced with high efficiency using a retroviral vector (MFG-GC) [27]. Analyses of the tissues of mice reconstituted with transduced BM revealed that the efficiency of gene transfer was essentially 100%. At 4 - 8 months post-transplant, BM, spleen, thymus and lymph nodes were completely repopulated by donor cells containing 1 - 2 copies of the transgene. Enzymatic analyses for GC activity in the tissues of transplanted animals showed that the activity was 4 - 5 times higher than control levels. In addition, we have performed secondary BM transplants with

the marrow obtained from the primary transplants described above. Secondary recipients were analysed for the presence and expression of the human transgene. Spleen colonies at 12 days post-transplant were all positive for the human transgene and the enzymatic activities in extracts of the colonies were increased 4 - 5 times, compared to control animals transplanted with non-transduced BM. The leukocytes of secondary recipients analysed for enzymatic activity in the blood 1 year after transplant showed that the transferred gene expressed GC at levels 4 - 5 times higher than that of control animals. This study demonstrated that it is possible to achieve long-term correction of murine BM stem cells by retroviral-mediated gene transfer. In humans, the correction of enzymatic activity by gene transfer to CD34+ progenitor/stem cells obtained from patients with Gaucher disease has also been accomplished [28-31].

These preclinical studies have laid down the foundation for clinical trials of gene therapy for Gaucher disease. Three clinical trials of HSC gene transfer and autologous transplantation without myeloablation for Type 1 Gaucher disease have been performed. Two trials employed a protocol consisting of transduction on autologous BM stroma in the presence of IL-3, IL-6 and SCF (with or without IL-1), protamine-sulfate and glucocerebrosidase-expressing viral supernatant for 3 - 5 days. In the first clinical trial, no gene-transduced cells engrafted in the patients [32], while in the second a low level of corrected cells (0.02%) was detected in the PB, which did not result in GC expression and clinical benefit [33]. In the third clinical study, we used a 1-day prestimulation of CD34+ cells in long-term BM culture medium containing IL-3, IL-6 and SCF and protamine sulfate, followed by two 2 h or one 4 h transduction with GC-expressing MFG viral supernatant, through the method of centrifugal enhancement [34]. Four patients received transplants of genetically modified cells. Transduction efficiency averaged 20% and enzymatic activity of GC in transduced CD34+ cells increased 10-fold over baseline. The average transduction efficiency in HPP-CFC was 9% and for one of the transductions, 29% of the colonies were marked. Total PB lymphocytes and CD34+ cells harvested from the PB showed presence of the GC transgene by PCR and increased GC enzymatic activity following transplantation. In one patient, the GC activity of total PBL rose to a level of as high as 80% of control and the transgene was detected in the three different FACS-sorted lineages of lymphocytes, polymorphonuclear leukocytes and monocytes. These results permitted a gradual withdrawal of ERT over 9 months. During this time and for an additional 5 months, the enzymatic activity in PBL remained substantially above deficient levels. Moreover, the dose reduction did not result in a decline in clinical status. However, over the next 12 months, clinical laboratory parameters slowly worsened and ERT was reinstituted. In the other three patients, no signs of toxicity were observed. However, the engraftment of transduced CD34+ cells was not persistent and the enzymatic activity was only marginally increased. We concluded that CD34+ cells from Gaucher disease patients can be safely transduced with a retroviral vector and transplanted in non-myeloablated recipients. In one patient, the engraftment of transduced cells and the correction of the enzymatic deficit persisted for more than 2 years and were accompanied by an apparent clinical benefit. We hypothesise that improvements to the gene therapy protocol may lead to consistent, long-term efficacy.

Several strategies can be used to increase the efficiency of gene therapy of HSC for Gaucher disease and other LSDs. First, improvements can be made to the retroviral vectors, by using oncoretrovirus-derived constructs with a better expression profile [35], or by using non-oncoretroviral vectors, such as the lentiviral vectors derived from HIV or other non-primate lentiviruses [36]. The main advantage of lentiviral vectors over the oncoretroviral vectors like the Murine Moloney Leukemia Virus (MMLV) vector, which have been widely used for gene therapy in experimental settings and in clinical trials, is the capacity to infect non-dividing cells [37,38]. These cells include human HSC [36,39] and terminally differentiated neurones [37,40]. Second, *in vitro* conditions that would increase the cycling of HSC leading to higher transduction efficiency can be explored [35]. These include GF stimulation and the use of cyclins, cyclin-dependent kinases and their inhibitors to act at the level of the intracellular regulators that control cell cycle [41]. Third, the BM microenvironment, which includes the stroma and molecules of the extracellular matrix, such as fibronectin, has been shown to enhance HSC transduction. It is therefore possible that once the biological mechanisms underlying the interaction between the HSC and the microenvironment have been elucidated, additional improvements will be derived by the use of this strategy. Fourth, liposomal pretreatment of retroviral supernatants has been shown to synergistically enhance the effect of centrifugal enhancement on transduction efficiency of CD34+ cells [42]. This procedure was readily transferable to a clinical setting (Swaney, personal communication) and thereby is a candidate for large-scale clinical use in future trials.

Another intriguing gene therapy strategy for Gaucher disease exists in the systemic delivery of GC by transplanted long-term expressing somatic cells that may secrete the enzyme in the circulation for macrophage uptake. Encouraging studies have shown that primary human myoblasts express and secrete GC *in vivo*; the secreted enzyme is then taken up by liver and BM macrophages [43,44].

3.2 Fabry disease

In response to the encouraging results of enzyme replacement therapy for Fabry disease [4], several groups have explored the feasibility of gene therapy for patients with this condition. Fabry disease is caused by a deficiency in the lysosomal hydrolase α-galactosidase A (AGA), which leads to accumulation of α-galactosyl-terminal lipids such as globotriaosylceramide (Gb3). The main pathological alteration is represented by systemic vascular occlusion causing cardiovascular, cerebrovascular and renal disease. Unlike other LSDs, there is minor

neuropathology, mainly affecting the dorsal root ganglia and the cells of the autonomic nervous system [45]. Similarly to Gaucher disease and other LSDs, overexpression of the lysosomal enzyme by gene-corrected cells results in secretion of the enzyme and its subsequent uptake by bystander cells [46,47]. Therefore, several gene transfer strategies have been employed to overexpress AGA, including viral and non-viral (plasmid-mediated) methodologies. The most impressive results have been obtained by transplanting retrovirally transduced BM mononuclear cells in a recently developed murine model of Fabry disease by Takenaka *et al.* [48]. This knockout mouse displays a complete lack of AGA activity, with accumulation of the substrate in the liver, kidneys and cultured fibroblasts [15]. AGA-deficient BM mononuclear cells were retrovirally transduced to produce AGA and transplanted in the AGA-deficient mouse model. Long-term increased AGA activity and decreased Gb3 storage levels were observed in the haematopoietic organs, liver, heart, lung and kidney of sublethally and lethally irradiated primary and secondary recipient mice. The correction was obtained in spite of a low percentage of marked cells. This lends experimental support to the hypothesis that even low levels of gene-corrected cells may be sufficient to achieve therapeutic efficacy in most LSDs; in Fabry heterozygotes, enzymatic levels of 10% of the normal value are compatible with a normal lifespan and quality of life.

Another successful preclinical gene transfer strategy for Fabry disease has been developed by Jung *et al.* using an adeno-associated virus (AAV)-derived vector [49]. AAV has been extensively used as a potential gene delivery vehicle [50,51] because it presents several advantages over the retroviral vectors. AAV is a single-stranded DNA virus that is nonpathogenic, can transduce both dividing and nondividing cells and achieve long-term expression of therapeutic genes in target cells. It is not pathogenic and is naturally replication-defective. Moreover, the removal of all viral coding sequences in the production of recombinant AAV minimises potential immune reactions against viral proteins, thereby lowering the risk of an inflammatory immune response significantly [50]. Using an *in vivo* direct viral delivery strategy, Jung *et al.* injected an AAV vector engineered to produce AGA under the EF1-α promoter into the portal vein of AGA-deficient mice [49]. A single delivery of the vector to the Fabry mouse liver was sufficient to restore AGA activity to levels necessary to correct the Gb3 storage. With the exception of kidney, this correction was seen in all visceral organs tested. More importantly, the glycolipid storage levels remained at near-normal levels for up to 5 weeks post-injection and persisted at levels 40 - 60% lower than the untreated Fabry animals for up to 6 months. This strategy of AAV vector delivery into a depot organ, such as liver, proved to be non-toxic and did not elicit a significant immune response in the mice. It could therefore be a valuable gene therapy option for Fabry disease and other LSDs.

3.3 Pompe disease

Glycogen storage disease Type II (GSD-II), also known as Pompe disease, is a fatal genetic muscle disorder caused by a deficiency of acid α-glucosidase (GAA). This enzyme defect results in lysosomal glycogen storage in multiple tissues with cardiac and skeletal muscles being the most seriously affected [52]. The clinical severity of the disease and the absence of any effective therapy (although the results of the clinical trials of ERT for Pompe disease appear promising [6,7]) have prompted the development of specific gene therapy strategies. Both *ex vivo* and *in vivo* gene delivery experiments have been conducted. In *ex vivo* experiments, fibroblasts and myoblasts from GSD II patients were infectable by retroviral and adenoviral vectors encoding for GAA [53,54]. The transferred gene was efficiently expressed and the *de novo*-synthesised enzyme reached lysosomes where it digested glycogen. The transduced cells secreted GAA that was endocytosed *via* the mannose-6-phosphate receptor into lysosomes of deficient cells and digested glycogen.

In vivo gene delivery approaches that targeted the muscles of GSD II animal models have only achieved limited results. When a replication-defective adenoviral vector encoding GAA was injected into the pectoral muscle of GSD II Japanese quails, only a local, short-term correction of the enzyme deficiency and glycogen storage was obtained [55]. Efficient, systemic correction of the muscle disorder was instead obtained after iv. injection of a modified adenovirus vector encoding human GAA in GSD II knockout mice [56]. Hepatic transduction and secretion of high levels of the precursor GAA proenzyme into the plasma of treated animals were observed. Subsequently, systemic distribution and uptake of the proenzyme into the skeletal and cardiac muscles of the GAA-knockout mouse was confirmed. As a result, systemic decreases (and correction) of the glycogen accumulations in a variety of muscle tissues was demonstrated. However, long-term studies will be necessary to determine whether the adenoviral vector may be able to sustain long-term expression of the transgene and whether immune responses my be generated in the animals. Moreover, it is not known if the improvement in the pathology resulted in improved muscle function in this study.

4. Gene transfer for LSDs with CNS involvement

4.1 Gene transfer and transplantation of BM cells

Gene therapy appears to be a valuable therapeutic option for LSDs with neurological involvement, due to the inability of ERT and BMT to improve the neuropathology of these disorders [57].

Gene transfer and transplantation of BM progenitors or mature blood cells such as macrophages may have a role in the treatment of storage diseases with CNS involvement. This strategy may be efficacious through several possible mechanisms:

- Degradation of storage material may occur by genetically-corrected enzyme-competent cells derived from the transplanted BM ('sink' effect), thereby reducing the possibility of accumulation of the storage material in brain.
- The transgene product (enzyme) may be secreted from BM-derived brain macrophages to neighbouring cells (glia).
- Circulating enzyme may be endocytosed by macrophages destined for the CNS.

A LSD that may benefit from gene transfer and transplantation of BM cells is MLD. MLD is an autosomal recessive disorder of sulfatide metabolism adversely affecting the formation and maintenance of myelin. Individuals with MLD are deficient in the activity of arylsulfatase A (ASA), a lysosomal enzyme that hydrolyses galactosyl sulfatide (cerebroside sulfate). This single gene defect gives rise to lysosomal storage and accumulation of sulfatide in the white matter of CNS and peripheral nerves and to a lesser extent in the visceral organs. It is believed that sulfatide-induced changes are responsible for the loss of myelination in the CNS and the range of ensuing neurologic deficits that often result in death.

We have shown that transduction of murine BM with a vector that carries the arylsulfatase A cDNA (MFG-ASA) resulted in highly efficient gene transfer, as demonstrated by the presence of vector DNA in 90% of spleen colonies 12 days after BMT [58]. The vector sequence was detected in macrophage cultures obtained from transduced donor BM cells and subsequently in the BM, spleen, lung, liver and brain of long-term reconstituted mice (4 months after BMT), indicating that BM-derived cells had migrated to and repopulated the viscera and the brain. Furthermore, the DNA signal was present in 100% of the spleen colonies of mice transplanted secondarily with BM from long-term reconstituted mice, indicating not only successful reconstitution but evidence of transduction of the pluripotent HSC in the primary recipients. Enzyme activity in the brains of mice 4 months after BMT was unchanged as compared to non-transduced control samples, probably as a result of the small number of BM-derived cells that enter the brain. However, approximately two out of three spleen colonies obtained from secondarily transplanted mice expressed arylsulfatase A above control levels, indicating that the MFG vector was continuing to express the transgene. More recently, Matzner *et al.* showed that 50% of ASA-deficient mice transplanted with BM cells retrovirally transduced with human ASA had long-term expression of ASA in several organs, including the brain [59]. The enzymatic activity in the brain reached 33% of the normal tissue level. Since the amount of enzyme delivered to the brain did not correlate with ASA serum levels, the authors speculate that the mechanism responsible for the gene transfer to the brain in their study may have been migration of BM-derived cells to the brain rather than endocytosis of serum ASA by endothelial cells.

Promising results were also obtained in a similar gene therapy strategy for MPS VII, a disorder caused by a genetic alteration of the GUSB gene. Transplantation of MPS VII BM cells retrovirally transduced to produce GUSB in MPS VII mice led to decreased substrate accumulation, despite low-level enzymatic expression in the liver, spleen and brain [60,61]. It is interesting to note that only mild conditioning (4.5 gray of total body irradiation) was necessary to achieve partial chimerism and metabolic correction of the disease. This finding was confirmed by Takenaka *et al.* in the aforementioned study of gene therapy for Fabry disease [48] and has clinical relevance. Although full myeloablation of patients with LSDs prior to transplantation would be an unacceptable method to enhance the engraftment of transduced cells due to its high toxicity, it is possible that low-dose pre-conditioning may be used in future clinical trials. No myeloablation was necessary in a gene therapy strategy using macrophages as target cells of gene therapy for MPS VII [62]. When either normal syngeneic macrophages or MPS VII macrophages retrovirally transduced with human GUSB were transplanted into the mice, GUSB was detectable histochemically *in vivo* at 38 days after transplantation and significant pathological improvement of lysosomal storage in the liver and spleen resulted. However, the main limitations of this strategy are the short lifespan of terminally differentiated macrophages, which can only sustain short-term correction, and the inability of the transplanted macrophages to reach the brain.

4.2 Intracranial injection of recombinant retroviral vectors

An alternative gene therapy strategy is represented by the direct delivery of the functional gene into the CNS. This may be achieved by intracranial injection of recombinant viral vectors or by implantation of genetically modified cells into the CNS. Direct gene transfer has been achieved with several classes of vectors including herpes virus, adenovirus, AAV, retrovirus and lentivirus-derived vectors. Herpes virus vectors have been used to deliver GUSB to the brains of an MPS VII mouse model following stereotactic injection. However, very few cells were transduced and they remained clustered near the injection tract [63]. More striking results have been obtained with AAV vectors and lentiviral vectors. A stereotactically-injected AAV vector containing the human GUSB cDNA could achieve a broad and sustained lysosomal enzyme delivery into the striatum of adult mice severely affected by MPS VII [64]. Similarly impressive results were obtained when an HIV-derived lentiviral vector was employed to reverse the pathology of the same MPS VII mouse model [65]. In mice receiving multiple injections of the GUSB-encoding lentiviral vector, complete correction or significant reduction of the pathology was observed in all histochemically-stained serial sections of the brain, suggesting disease regression in the entire brain. A lentiviral-vector *in vivo* gene therapy approach was also used to correct the neurological lesions of a mouse model of MLD [66]. A stereotactic injection of the ASA-encoding lentiviral vector into the hippocampal fimbria of the

mice determined sustained expression of the enzyme throughout most of the injected hemisphere, with rescue of neurones from degeneration and long-term protection from neuropathology.

4.3 Implantation of genetically modified cells into the CNS

Gene transfer into the brain can also be achieved through neurotransplantation of genetically modified cells. Transduced fibroblasts and myoblasts have been implanted in the brain with limited results. Taylor *et al.* observed decreased lysosomal storage in the adult MPS VII mouse brain in the vicinity of grafts of retroviral vector-corrected fibroblasts secreting high levels of GUSB [67]. The oligodendrocyte would be the optimal target for gene therapy of MLD. Oligodendrocytes have been transplanted into mouse models of demyelinating disease and shown to be able to survive, migrate and myelinate in the brain [68,69]. Mouse and rat neural progenitor cells have been identified which have the potential to differentiate into glial or neuronal cell types [70,71]. These cells also retain functional properties of neuronal and glial cells *in vivo*. When the CG-4 cell line, a rat oligodendrocyte progenitor cell line expressing a LacZ marker gene, was transplanted into the spinal cords of myelin-deficient rats, expression of LacZ was obtained in cells that had migrated along the spinal cord and showed the ability to myelinate neighbouring neurones [72]. Snyder *et al.* injected retrovirally-transduced murine neural progenitor cells (C.17) expressing the GUSB gene into the brains of newborn MPS VII mice [73]. The donor cells expressed the GUSB transgene in both neuronal and glial cell types and there was evidence of a reduction of lysosomal storage in host cells, presumably by a cross-corrective mechanism. More recently, in a gene therapy strategy aimed at correcting Tay-Sachs disease, multipotent neural cell lines transduced to express the β-hexosaminidase α-subunit cDNA were transplanted into the brains of both normal fetal and newborn mice. Engrafted brains, analysed at various ages after transplant, produced substantial amounts of human β-hexosaminidase α-subunit transcript and protein, which was enzymatically active throughout the brain at a level reported to be therapeutic in Tay-Sachs disease [74]. These data point to the intriguing possibility of establishing a human cell line which is both transplantable to the CNS and which maintains the functional properties of oligodendrocytes. Such a cell line would offer the potential of treating MLD by gene transfer to and transplantation of, the pathologically important cell type. In recent studies, both non-autologous neural progenitor cells and fibroblasts genetically engineered to produce GUSB were enclosed in immunoisolating microcapsules and transplanted into the CNS of MPS VII mice, with improvement of the neuropathological lesions [75,76]. This strategy may overcome the immunological rejection of non-autologous cell grafts.

Finally, other cell types like amniotic epithelial cells and BM stromal cells (otherwise named mesenchymal stem cells or MSC) have been explored as gene delivery vehicles to the CNS. An amniotic epithelial cell line engineered to produce GUSB was used as donor cell source in a cell-mediated gene therapy of MPS VII with success [77]. After transplantation of the cells in the brain of MPS VII mice, extensive GUSB activity was detected in the hemilateral hemisphere of the brain, with pathological improvement of lysosomal storage in areas distant from the injection site. MSC are particularly attractive gene delivery vehicles for a number of reasons. First, they have an extensive differentiation potential, including the capacity of being precursors of non-haematopoietic, non-mesenchymal-derived cells (reviewed in [78]). Studies involving direct injection of MSC into the rodent brain showed that the cells were able to engraft, migrate and differentiate into astrocytes [79,80]. Second, MSC can be easily isolated and culture-expanded [81]. Third, MSC can be efficiently transduced with retroviral vectors. Marx *et al.* reported transduction efficiencies of up to 80% using a murine stem cell virus-based vector [82]. In a neurotransplantation study, gene transduced MSCs were injected into the striatum of a rat model of Parkinson's disease [83]. The cells were able to engraft and survive for approximately 3 months in the brain of the animals. However, expression of the transgenes ceased at about 9 days, an observation consistent with reports from other laboratories in which retroviruses similar to those employed in the study were used to express transgenes in the brain.

4.4 Neonatal transplantation

Daly *et al.* showed that the need for invasive injections of viral vectors or transduced cells in the brain may be obviated if gene therapy is performed in the neonatal period [84]. An iv. injection of GUSB-encoding AAV vector in neonatal MPS VII mice resulted in therapeutic levels of GUSB expression by 1 week of age in liver, heart, lung, spleen, kidney, brain and retina. GUSB expression persisted in most organs for the 16-week duration of the study at levels sufficient to either reduce or prevent completely lysosomal storage. Of particular significance, neurones, microglia and meninges of the CNS were virtually cleared of disease. The authors hypothesise that the AAV vector gained entry into the brain either because of an incompletely formed BBB in neonate mice, or because of the higher per kilogram dose of virus administered, compared to doses previously delivered to adult mice.

5. Antenatal gene transfer

In many neurodegenerative storage disorders with clinical onset during infancy, there is evidence to suggest that the abnormal CNS storage of the substrate begins *in utero*. For instance, an increased concentration of sulfatide has been shown in the cerebellum, brain stem and spinal cord of a 24-week old foetus with MLD [85]. Excess of sulfatide has also been noted in myelin isolated from another foetus with MLD [86]. In a series of electron microscopy studies in foetuses (gestational age 12 - 22 weeks) with Tay-Sachs disease, Adachi

et al. found membranous inclusions in the anterior horn cells, spinal ganglia, retina and pituitary gland [87]. In the neuronopathic variants of Gaucher disease (Type 2 and Type 3), it is possible to hypothesise that glucosylceramide accumulation in brain produces dysfunction in surrounding cells long before discrete pathologic changes are observed [88]. Advanced pathological alterations in fetal tissues, including the CNS, have been documented in the acute neuronopathic variant of Gaucher disease (Type 2) [89], which is characterised by onset of severe disease in the foetus [90]. It is likely that these disorders not only cause degeneration of preformed neurones but also interfere with normal developmental events, with potentially irreversible consequences. BMT performed in the postnatal life is usually unsuccessful in reversing the neurological complications of these disorders [57]. The neurodevelopmental outcome in these patients will therefore be greatly improved by early treatment in the foetus. Other disorders that have been corrected by postnatal BMT such as Type 1 Gaucher disease [91] and Maroteaux-Lamy syndrome (MPS VI) [92] would also be candidates for prenatal transplantation of genetically modified cells. Intra-uterine gene transfer should be facilitated by the immunologically permissive environment of the early gestational foetus. The clinical experience of intra-uterine haematopoietic stem cell transplantation for LSDs has, however, been dismal. In five attempts to transplant foetuses with LSDs, there was little or no engraftment of donor cells and no clinical benefit (reviewed in [93]).

Two intra-uterine gene therapy strategies have been evaluated in animals: the transfer of genetically engineered HSC and the direct injection of the vector. The gene-engineered HSC transplantation approach resulted in the long-term transfer and expression of the transgene at low efficiency in sheep and monkeys. In a canine model of human α-L-iduronidase deficiency (MPS I), Lutzko *et al.* have performed *in utero* adoptive transfer of iduronidase-transduced MPS I marrow cells into pre-immune fetal pups. The iduronidase-transduced primitive haematopoietic progenitors engrafted in the fetal recipients but no iduronidase enzyme nor proviral-specific transcripts were detected in blood or marrow leukocytes of any MPS I dog [94]. Higher transduction efficiency will need to be achieved for this strategy to be effective for the treatment of human LSDs. The results of the experiments of direct injection of the vector were more encouraging. Inoculation of retroviral supernatant into the liver of fetal rats successfully transduced fetal liver HSC and in pre-immune fetal sheep, the direct injection of viral vector into the peritoneal cavity led to long-term expression of the transgene in all tissues of the animals analysed, including the brain (reviewed in [95]). These results are of particular interest for the development of intra-uterine gene therapy approaches for LSDs with neurological involvement.

6. Conclusion and expert opinion

The medical care of LSD patients has been revolutionised by the development of effective therapeutic strategies, wherever only supportive therapy was available. ERT is becoming a reality for many LSDs and the indications for BMT are being extended, as this therapy becomes progressively safer. Moreover, substrate deprivation therapy is emerging as a promising treatment for glycosphingolipidosis such as neuronopathic Gaucher disease [96] and Sandhoff's disease (GM2 gangliosidosis) [97]. However, each of these treatments has disadvantages that substantially limit its application. Most notably, the attempts at reversing the neuropathological manifestations of many LSDs have been disappointing. This provides a compelling rationale for the development of gene therapy strategies aimed at preventing or treating the devastating systemic consequences of the enzyme deficiencies. Indirect gene therapy strategies have been devised to efficiently transduce a number of transplantable cell targets. Haematopoietic, neural and mesenchymal stem cells are the most appealing cell subsets because of their extensive self-renewal and differentiation potential. However, their biological behaviour *in vitro* and *in vivo* has only been partially elucidated. Optimal *ex vivo* culture conditions which may allow preservation of the stem cell phenotype while promoting efficient gene transfer have not yet been found for haematopoietic and neural stem cells. The newly devised AAV and lentiviral vectors offer definite advantages in this arena, due to their capacity of infecting non-dividing cells (such as most stem cells) at high efficiency. However, they raise several safety concerns, since their immunogenic, oncogenic and infectious potential *in vivo* has not yet been fully evaluated. MSC are readily expandable and transducible *in vitro* but their behaviour *in vivo* is still unpredictable, due to the lack of extensive clinical experience. Direct viral injection may be the preferred gene therapy approach whenever systemic expression, or access to biological sanctuaries (such as the CNS) are warranted. Again, promising results have been obtained in animal models of LSDs using AAV and lentiviral vectors but more preclinical testing is necessary before clinical trials will be initiated.

In the last years, gene therapy has been under strong critical scrutiny after more than 400 clinical trials failed to show any consistent clinical efficacy and the tragic death of a patient in an ornithine transcarbamylase clinical trial shook the scientific community and the public confidence [98]. These events have overshadowed the significance of a few promising results from some recently concluded clinical trials for X-linked severe combined immune-deficiency (SCID) [99], chronic granulomatous disease (CGD) [100,101], haemophilia B [102] and transfer of multi-drug resistance (MDR) [103]. These studies have shown that even low levels of gene marked cells may be sufficient to achieve clinical efficacy. However, in the case of X-linked SCID, where the clinical benefit was more convincingly demonstrated, the corrected cells had a selective advantage over the diseased counterpart. In most LSD, the corrected cells would not selectively outgrow the enzyme deficient cells. The question is therefore whether or when these positive results will be extended to the gene therapy of LSD.

Since the spectrum of the diseases is ample and heterogeneous, it is important to draw some distinctions. In diseases such as Type 1 Gaucher disease, where a clinical trial showed sustained correction of the peripheral blood cells with apparent clinical benefit in one patient [34], a definitive gene therapy cure can be foreseen in the near future. Only incremental improvements of the gene therapy strategy employed in the study may be needed to achieve higher, longer-term transduction efficiency. This may also be the case for Fabry disease, for which an informative animal model is available [15]. This Fabry mouse model has already allowed an efficient gene therapy strategy using murine BM cells [48]. In the case of LSDs with neurological involvement, a gene therapy cure in the next few years seems an unrealistic expectation. The two main technical problems to overcome are the need to gain access to the CNS and to intervene before neurological complications have ensued. Strategies of stereotactic injection of viral vectors or gene-marked cells are being developed, but the results have been controversial. Moreover, optimism in the neurotransplantation research field has partly subsided after a clinical trial for Parkinson's disease showed that transplantation of embryonic dopamine neurones was partially effective only in young patients and elicited severe adverse effects in 15% of the transplanted patients [104]. Additional larger studies and the results of the gene therapy trial of neurotransplantation of nerve growth factor-producing cells for Alzheimer's disease conducted at the University of San Diego, will hopefully provide more insight into this gene therapy approach. Antenatal (*in utero*) gene transfer and transplantation are appealing strategies to prevent the neuropathology in LSDs but technical problems such as cell dosing and timing of the injection and ethical problems must be solved before this will be considered a feasible gene therapy approach.

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